Molecular determinants of human renal clear cell carcinoma cancer risk

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KUMARI VANDANA RANI

Faculty of Medicine and Health Sciences

School of Veterinary Medicine and Science

University of Nottingham

U.K.

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Abstract

Hypoxia-related pathways play key roles in the mechanism that lead to development of acute kidney injury (AKI) and clear cell renal carcinoma (ccRCC). This dissertation used a pig model of AKI and a mouse model of ccRCC to compare the molecular mechanisms of these two disease processes to determine whether AKI may contribute to ccRCC risk.

In the present study we first used next generation sequencing to identify single nucleotide variants (SNVs) and splicing effects in the mouse TRACK ccRCC model relative to wild-type control mice. This analysis identified multiple variants in TRACK mice, including an intronic variant in the *NDUFA4L2* locus predicted to alter splicing and a coding variant in the *CES3* gene. We analyzed the prevalence of mutations and differential expression in these genes in human ccRCC genomic data. Our study has identified a novel *CES3* mutation in mice over-expressing HIF-1α and several other genes like *Hilpda, Zbtb38, Cdc58, Plekhm2* and *Aldoa* illustrated splice junctions variations. The association of these genes with ccRCC have to be investigated. Our results showed over expression of *MYC* and *ANXA2* gene in both AKI and ccRCC model and in human ccRCC as well.

Secondly, we used RNA sequencing to compare the transcriptome of kidney samples from the pig and mouse models of AKI and ccRCC respectively. This analysis identified a network of genes deregulated in both AKI and ccRCC. The expressions of these genes were further analyzed in the cancer genome atlas patient ccRCC (KIRC) RNA sequencing dataset. Collectively these analyses have identified increased expression of MYC as an important driver of the pathogenic processes in both AKI and croc. Further retrospective clinical studies are now warranted to investigate whether AKI predisposes to ccRCC in people.

Abbreviations

AKI:	Acute kidney Injury
AKIN:	The Acute Kidney Injury Network
AKIN:	Acute Kidney Injury Network
ALAT:	Alanine aminotransferase test
ALL:	Acute lymphoblastic leukemia
ALP:	Alkaline phosphatase
ANP:	Atrial natriuretic peptide
ANXA2:	Annexin A2
ARE:	Antioxidant response element
ARF:	Acute renal failure
ASAT:	Aspartate aminotransferase test
ATM:	Ataxia telangiectasia mutated
ATR:	Ataxia–telangiectasia and Rad3 related
ATN:	Acute tubular necrosis
BH:	Benjamin-Hochberg
BHD:	Birt-Hogg-Dubé syndrome.
BWA:	Burrows–Wheeler-Transform
ccRCC:	Clear cell renal cell carcinoma
CD:	Cluster of differentiation
CES3:	Carboxylesterase 3
chRCC:	Chromophobe renal cell carcinoma
CKD:	Chronic kidney disease
CSCs:	Cancer stem cells

CT:	Collecting duct
DCT:	Distal convoluted tubule
DDR:	DNA damage response and transformation grade
DEG:	Differential Gene expression
DSB:	Double stranded breaks
ECM:	Extracellular matrix
EPO:	erythropoietin
ERBP:	European Renal Best Practice
ESRD:	End stage renal disease
ESRF:	End stage renal failure
FH:	fumarate hydratase
FPKM:	Fragments per kilobase of transcript per million fragments
GFR:	Glomerular filtration
GO:	Gene annotation
GT:	Gamma-glutamyl transpeptidase
HIF:	Hypoxia inducing factor
HLRCC:	Leiomyomatosis renal cell cancer
HPRC:	Hereditary papillary renal carcinoma
HR:	Hpmologous recombination
HRG:	Histidine-rich glycoprotein
ICNARC:	Intensive Care National Audit Research Centre
ICU:	Intensive care units
IGV:	Integrated genome viewer
IHC:	Immunohistochemistry
IM:	Intermediate mesoderm

IRI: Ischemic renal injury

KDIGO: Kidney Disease: Improving Global Outcomes

KIRC: Kidney Injury research network ccRCC

LD: Lactate dehydrogenase

LH: Henle's loop

LOH: Loss of heterozygosity

LOX: Lysyl oxidase

MM: multiple myeloma

MMP: matrix metalloproteinase

NAG: N-acetyl-β-glucosamineidase

nccRCC: Non-clear cell carcinoma

NDUFA4L2: NADH Dehydrogenase (Ubiquinone) 1α subcomplex 4-like 2

NGAL: Neutrophil gelatinase-associated lipocalin

NHEJ: non-homologous end joining

NQO1: NAD(P)H:quinone oxidoreductase

PAX2: Paired box 2

PCT: Proximal convoluted tubule

PDGF: Platelet-derived growth factor

PHD: Prolyl hydroxylases

pRCC: Papillary RCC

RBP: Retinol binding protein

RCC: Renal cell carcinoma

RIFLE: Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease.

RPKM: Reads per kilobase of transcript per million fragments

SIX2:	Sine oculis homeobox 2
SNVs:	Single nucleotide variants
TCGA:	The cancer genome atlas
TGFα:	Transforming growth factor alpha
TRACK:	The transgenic cancer of the kidney
UICC:	Union for International Cancer Control
VEGF:	Vascular endothelial growth factor
VEP:	Variant Effect Predictor
VHL:	von Hippel Lindau
WT1:	Wilms tumour 1

Chapter 1: Introduction

1.1 Kidney

In vertebrates, several essential regulatory functions are performed by kidney. These functions are underpinned by a complex anatomical architecture which facilitates the physiological and molecular functions of the kidney. The mature human kidney measures approximately 12x6x2.5 cm^3 (Arooj et al., 2011; Sm Nurul Hassan, 2013; Murlimanju et al., 2014). Most individuals have two kidneys, although, individuals with one kidney or a fused, "horseshoe" kidney is not rare (Mirzazadeh and Richards, 2011; Nikumbh et al., 2014; Pupca et al., 2014; Rao and Battula, 2015).

The kidney is an essential organ associated with the maintenance of the electrolytes, acid-base equilibrium and blood pressure in addition to the urine production by filtration and elimination of all the soluble wastes (e.g. urea and ammonia). In order to produce about one litre of urine in a day it filters out about 1700 litres of blood (Fritzbefatsrg, 1984;Guyton,1996; Zaman et al., 2011; Barron,2012; Ahmed, 2015). During filtration process useful components such as water, amino acids and glucose is reabsorbed (Guyton, 1996).

1.2 Location and Structure

Kidneys, as a paired organs located in the posterior region of the visceral cavity behind peritoneum. The "bean" shaped structure of the organ provides two sides of the kidney; the convex side positioned laterally whereas concave side is placed medially. Anatomically the left kidney are slightly superior to the right kidney since on right side the lobes of liver are bigger compare to left side. The kidney is protected internally by its surrounding fat named as Perirenal fat whereas ribs and back muscles keep safe from external shocks.

Outermost covering of kidney (renal capsule) is made up of fibrous connective tissue that act as firm outer shell and internally separated into three regions: outer cortex, middle medulla and renal pelvis in region of hilum. Renal hilus an indentation on the concave side of kidney helps to arrange space for distribution of renal arteries/veins and ureter ducts entering into the kidney. Inside kidney, the seven cone-shaped structure called as renal pyramids extend from medulla region to the renal cortex and each apex of the cone combine to form a small tubular structure termed minor calyx which collect urine. Further, many minor calyx unite to form major calyx and these unite to form a hollow renal pelvis. The renal pelvis drain urine into ureter after it came out of the kidney at hilus (Vander et al., 1985).

1.2.1 The Nephron

The nephron epitomizes the microscopic structural and functional filtration units of kidney which possess approximately more than $1x10^6$ nephrons. It comprises of the renal corpuscle and renal tubule which are responsible for the reabsorption or secretion of components. The renal corpuscle is characterized by presence of fine glomerular capillaries and capsule. The renal corpuscle is characterized by presence of fine glomerular capillaries and a hollow cup-shaped capsule which is also called as Bowman's capsule. The glomerulus is physiologically very important as it increases the surface area of blood in contact the wall of blood vessel. The special epithelial cells, known as podocytes, act as thin filter within the endothelium to filter urine from the blood of glomerular capillaries. The renal tubule comprises 3 parts: proximal convoluted tubule (PCT), distal convoluted tubule (DCT) and collecting duct (CT), all these together concentrates urine by recovering valuable solutes from the urine and allows its passage towards the renal pelvis coming via glomerular capsule. The urine first pass through proximal convoluted tubule (PCT) where reabsorption of large

quantity of water and nutrients take place then urine pass through Henle's loop (LH), distal convoluted tubule (DCT) and finally from DCT of various nephrons. Further, it enters into collecting duct (CT) and finally passes resolute urine into the renal pelvis via the renal medulla (Guyton, 1996).

1.3 Kidney Functions

To maintain the homeostasis of entire body through regulation of acid-base equilibrium, regulation concentrations of electrolytes, volume of fluid, blood pressure are the prime functions of Kidney. and it also exhibits an endocrine functions (DeFronzo et al., 2011; Brown ad Wagner, 2012; Raghavendra et al., 2013; Cersosimo et al., 2014; El-Sharkawy et al., 2014; Hamm et al., 2015).

1.3.1 Filtration, Reabsorption and Secretion

Three crucial mechanisms accomplished in the kidney nephrons are filtration, reabsorption, and secretion. Renal corpuscle involves infiltration process in which larger components (which includes cells as well as proteins) are sieved out from the blood to produce ultra-filtrate. The kidney produces approximately 2 litres of urine by reabsorbing a large percentage of ultra-filtrate which is usually 180 litres a day - (Gilbert et al., 1992; Guyton, 1996).

1.3.2 Water Homeostasis

The quantity of water in the body is controlled by kidney through reabsorption process that leads to production of concentrated urine and it helps to preserve water in the body. The normal plasma osmolarity is retained by reabsorption of water which is enhanced under the influence of hormones, aldosterone and antidiuretic hormone (ADH) (Gilbert et al., 1992; Guyton, 1996). ADH stimulates the production and

insertion of water channel proteins, aquaporin-2(AQP2) present in the collecting ducts to allows passage of water from urine into tubule cells and into blood, whereas aldosterone increases of sodium and chloride ions which lead to more movement of water into blood via osmosis (Kanikowska et al., 2009; Gomes et al., 2009).

1.3.3 Acid/Base Homeostasis

Acid-base homeostasis is maintained by kidney through regeneration of bicarbonate ions (HCO3-) in urine and excretion of H^+ ions and certain acid anions into urine. During filtration period both ions are filtered from the blood, however selectively bicarbonate ions are absorbed in the tubule cells and hydrogen ions are allowed to be excreted in urine. As soon as the physiological pH of blood decreases i.e. blood becomes more acidic, the tubule cells may actively secrete hydrogen ions into the urine (Gilbert et al., 1992; Guyton, 1996).

1.3.4 Electrolyte Homeostasis

Sodium and potassium (Na^+, K^+) are important for various physiological activities such as functioning of neuron, regulation of blood pressure, contraction of muscles, and regulation of volume of blood. In excess of ninety-nine percent of sodium and approximately sixty to eighty percent of the potassium ions are reabsorbed in PCT and LH (Gilbert et al., 1992; Guyton, 1996; Palmer and Schnermann 2014). In addition, kidney improved the calcium ions under the influence of parathyroid hormone in response to very low level of calcium in blood.

Many other electrolytes including chloride, calcium and magnesium are vital for establishment of neuron's electrical potential of and muscle cells. Likewise, metal

cations including magnesium, sodium and potassium are important muscle contraction, neurotransmitter release by neurons and for proper function of enzymes that require ATP, DNA, and RNA (all containing phosphate compounds).These elements are predominantly reabsorbed by proximal convoluted tubule and loop of Henle (Gilbert et al., 1992; Guyton, 1996).

1.3.5 Renal endocrine functions

The kidney also plays role as endocrine gland by producing the hormone erythropoietin and calcitriol. The peritubular cells of the capillaries produce erythropoietin under hypoxic condition, which enhances the production of RBCs that to increase oxygen transport. The production of EPO is associated to the level of oxygen level (Gilbert et al., 1992; Guyton, 1996). The proximal convoluted tubule (PCT) has the potential to produce calcitriol that is an active form of vitamin D, using inactive form of vitamin D and then move from kidneys to intestine via the bloodstream, where it help in calcium absorption from food present in lumen of intestine.

Other hormones produced elsewhere but influence the function of kidneys: Antidiuretic hormone (ADH, also called as Vassopressin) is secreted by hypothalamus in response to reduced volume and increased osmolarity of the blood. Likewise Angiotensin II is synthesized by hepatocytes and can to be transformed to active form by the enzymes (renin and angiotensin) and influence absorption of sodium and calcium ions along with water in the PCT (Gilbert et al., 1992; Guyton, 1996).

In response to Angiotensin II, Aldosterone is produced in the adrenal cortex which help in balancing of ions (Na^+, Cl^- and K^+) by stimulating target cells of nephron and

collecting duct. Another Atrial natriuretic peptide (ANP) produce by the cardiac cells promotes blood flow into the glomerular capsule and renal tubules by increase in the glomerular filtration rate. Thus the main functions associated with ANP include increased excretion of sodium ions and water into urine, reduce volume of blood and blood pressure (Gilbert et al., 1992; Guyton, 1996).

1.4 Developmental Physiology

Initial development of kidney can be observed as result of successive morphogenetic steps firmly controlled in time and space. Apart from common development processes for other organs like interactions of epithelial and mesenchymal cells, branching morphogenesis, maintenance of stem & progenitor cells and differentiation of cells, their migration, defined pattern of cell division, and interactions of ECM (cell-extracellular matrix) in kidney development have some unique events. These events include highly specialized structures differentiation like glomerulus formation and mesenchymal-epithelial transition (MET) to form the nephron (Quaggin and Kreidberg, 2008, Costantini and Kopan, 2010).

The mammalian kidney develop by nephrosis i.e. development from intermediate mesoderm. The three development phases that follow one another include formation of pro-, meso-, and meta- nephros (Carlson, 2004). The epithelium of nephron as well as collecting ducts are known to originate from intermediate mesoderm during the process of embryogenesis, however, the source of development of vasculature is different. The process of organogenesis of kidney begins with the induction of pro-nephros and proceeds till the accomplishment of meta-nephros (Kuschert et al., 2001, Bouchard et al., 2002).

1.4.1 The genes involved in specifying the kidney. Are these genes important in ccRCC?

A limited number of genes are identified which can influence the pro- and mesonephros formation in advanced vertebrates Bouchard (Stec et al., 2009b). However, paired domain transcription factors such as *Pax2* and *Pax8* have been identified to be important for specifying the nephric lineage (Bouchard et al., 2002).

1.4.2 Stem and Progenitor cells

Ability of indefinitely proliferation is the characteristic feature of cancer cells (Hanahan and Weinberg, 2011a). There is resemblance between self- renewal property of stem cells and cancer cells which generate the concept of cancer stem cells (CSCs) (Reya et al., 2001). Mierau and colleagues reported that nephroblastomas regenerate normal embryonal kidney that are ultrastructurally and histologically similar (Mierau et al., 1987). In addition, it also mimics embryonal kidney (8 weeks of gestation) in overall gene expression (Dekel et al., 2002). Multiple interfaces among the ureteric bud, the compressed metanephric mesenchyme around the ureterical buds along with the stromal mesenchyme leads to normal nephrogenesis and further mature nephrons results as an outcome of differentiation in condensed metanephric mesenchyme (Saxen and Sariola, 1987, Dressler, 2006).

The multipotent cells of the metanephric mesenchyme give rise to all cell types of the nephron by the process of differentiation (Herzlinger et al., 1992; Qiao et al., 1995; Al-Awqati and Oliver, 2002). Multiple genes viz *WT1* (Wilms tumour 1), *HOX11* (homeo box11), *PAX2* (paired box 2), *SIX2* (sine oculis homeobox 2), and *EYA1* (eyes absent homolog 1) are essential for precise development of the metanephric kidney (Wellik et al., 2002; Schwab et al., 2003; Metsuyanim et al., 2008; Patel and Dressler;

2013). The first gene was *WT1*which showed genetic link between embryogenesis and tumourigenesis (Huff, 1998; Discenza etal., 2003) and later on *PAX2* have been recognized in the process of the development of nephroblastomas (Maschietto et al., 2011). *SIX2* gene was identified in the metanephric kidney, which morphologically similar to blastema in nephroblastomas and have vital role in renal development (Huff, 1998; Kobayashi et al., 2008; Sehic et al., 2012) and controls renal progenitor cells that are dedicated to epithelial differentiation (Self et al., 2006; Kobayashi et al., 2008; McLaughlin et al., 2013; Adli, et al., 2015).

1.4.3 Cancer stem cells

Low occurrence in cell culture systems as well as in tissue samples are the main hindrance in characterization of adult stem cells. Recent research has been successful in characterizing and identifying genes markers of stem cells (Tabrizi, et al., 2013). Numerous potential markers comprising *Oct-4, Sox2, Thy1, Cripto, FGF4, A zfp-42 (Rex*-1) and *Lefty* of human embryonic and stem cells adult stem cells have been identified (Abeyta et al., 2004; Nakatake et al., 2006; Kashyap et al., 2009; Calloni et al., 2013). Bussolati et al. used cell sorting to identify a group of tumour-initiating cells with a mesenchymal marker CD105. The cells account for less than 10% of the tumour mass which are expressing mesenchymal marker CD105 which expressed mesenchymal stem cell markers like vimentin CD29,CD44, CD73, CD90and CD146. In addition, it also expressed embryonic renal marker *Pax2* along with embryonic stem cells markers like Oct4, Nestin, Nanog and Musashi. CD105 expressing cells may signify a tumour-initiating cell with mesenchymal features which may have origin from local renal stem cells. Remarkably, CD105 expressing cells do not express CD133 which exist in the tubular compartment progenitors of adult kidney (Bussolati et al., 2005, Sallustio et al., 2010, Bussolati et al., 2012). In renal clear cell

carcinomas, CD133+/CD34 cells isolation and characterization revealed the existence of cells with particular progenitor functions. These cells have aptitude to differentiate into epithelial and endothelial but lost the tumour-initiating ability (Bruno et al., 2006).

1.4.4 Identification and Isolation of putative kidney stem cell

Stem cells have been identified in different organs such as bone marrow, gastrointestinal mucosa, liver, brain, prostate, and skin (Alison et al., 2001, Bernard-Kargar and Ktorza, 2001, Forbes et al., 2002, Morrison et al., 1999, Wright, 2000). In kidney, there are indications that stem cells exist in the metanepheric mesenchyme and differentiate into all other cell types (Herzlinger et al., 1992, Oliver et al., 2002, Lindgren et al., 2011, Hansson et al., 2014). Other putative candidate stem cells in the adult mammalian kidney have been identified from renal carcinomas using different identification methods (Hansson et al., 2016, Maeshima et al., 2003, Oliver et al., 2004, Maeshima et al., 2006, Kitamura et al., 2005). In the kidney different methods with their own limitations identify resident progenitor cells from differentiated adult cells are (1) detection of label-retaining properties (so-called label-retaining cells) due to low proliferative activity of stem cells, (2) detection of Hoechst dye extrusion via ATP binding cassette transporters by fluorescent-activated cell-sorting analysis (Challen et al., 2006, Addla et al., 2008), (4) stem cell marker expression analyses (CD133, CD24, CD106, stem cell antigen-1, and transcription factors specific to stem cell (*Oct-4* and *Bml-1*), and (5) genetic reporter systems, (Wilson and Thompson, 2007) selective culture conditions (Bertoncello and Williams, 2004).

1.4.5 Renal regeneration

There is evidence that stem cells can proliferate, dedifferentiate, and ultimately re-line shed kidney tubules, re-establishing the structural and functional veracity of the kidney (Bacallao and Fine, 1989, Safirstein, 1999, Maeshima et al., 2002, Safirstein et al., 1990 1994). The molecular events involved in the renal regeneration have now been characterized, and success has been achieved in expediting the process of repair in the experimental models as well as in humans. But classically the kidney is regarded as an organ incapable of true regeneration although this remains largely unexplored. It has a noteworthy capability to survive injury and re-establish function regardless of a limited turnover of cells under physiologic conditions. The shedding of 68,000–72,000 cells/hr from the kidney (Prescott, 1966) and the documented renal repair that follows damage propose that there is constant cellular renewal in the mammalian kidney (Witzgall et al., 1994, Challen et al., 2006, Little and McMahon, 2012, Rinkevich et al., 2014).

Two different approaches either non-transgenic (Poulsom et al., 2001, Gupta et al.), or transgenic (Duffield et al., 2005, Kale et al., 2003, Lin et al., 2005) were shown by several groups for the tubular compartment that bone marrow derived stem cells (BMSCs) were found in the tubular epithelium following injury. The lineage tracing experiments showed tubular regeneration occurred from intrinsic surviving tubular epithelial cells (Miyazaki et al., 2014) Renal Progenitor Cells in different sections of kidney to recover from injury are as 1) in tubules are CD106 (vascular cell adhesion molecule 1) with additional low proliferation CD133 + , CD24 + STCs (CD106) from CD133 + , CD24 + PECs (CD106 + (Angelotti et al., 2012) 2) glomerulus stem cell markers CD133, CD24 and the podocyte protein podocalyxin (PDX) (Ronconi et al., 2009) .

1.5 Acute kidney Injury (AKI):

1.5.1 Definition and staging of AKI, Estimation of kidney function:

Acute kidney injury may be broadly explained as "an unexpected and continued deterioration of kidney functions". Clinical signs include a sudden drop in the rate of rate glomerular filtration (GFR), resulting in loss of equilibrium in electrolytes, acid base instability in volume of extra cellular fluid, inability to excrete nitrogenous waste products and often reduced urine output (Reya et al., 2001). Till the date confusion is going on like how best to assess kidney function include what markers are that best reflect it, and what values of these markers distinguish normal from abnormal kidney function.

More than 35 definitions of AKI have been stated in the literature (Angelotti et al., 2012). The Acute Dialysis Quality Initiative to promote a reliable and stable definition of AKI for assessment of study results convened an international consensus panel in 2002 and offered the RIFLE criteria for use in adversely ill adults (Witzgall et al., 1994, Poulsom et al., 2001). The various changes observed in AKI when compared to the patients baseline kidney functions is utilized by the RIFLE to classify AKI in increasing severity of 5 categories: three ranks of enhanced severity Risk (R), Failure (F), Injury (I) and two attributes to variable functional loss of kidney (L), and End-stage kidney disease (E).The changes in filtration rate of glomerulus from a baseline and urine output are designated the first three severity levels (Risk, Injury and Failure).

The severity spectrum of AKI is broad, starting with risk of patients due to genetic or clinical risk factors, proceeding to renal injury or dysfunction and finally resulting into acute renal failure (ARF). It can represent as elusive structural and biochemical

changes, minimal elevations in serum creatinine, or anuric renal failure (Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Working Group, 2012). AKI can also arise as a comorbidity in critically ill children and adults (Duffield et al., 2005, Gupta et al., 2002) and increases risk of mortality in these patient populations independently (Kale et al., 2003, Duffield et al., 2005, Miyazaki et al., 2014, Lin et al., 2005). Also AKI can arise during acute hospitalization. However patients experiencing an AKI do usually require dialysis treatment.

Over the years there has been improvement in the usage of terminologies for AKI still currently three definitions are in practice (Table 1.1); 1) The Acute Dialysis Quality Initiative "RIFLE" criteria (Kitamura et al., 2005) that show risk, injury, failure, loss and ESRD 2) "AKIN" (Acute Kidney Injury Network) criteria (Angelotti et al., 2012) and 3) "AKI (Bonventre)" criteria. In 2012 the international guideline group published the AKI guidelines by name Kidney Disease: Improving Global Outcomes (KDIGO) that has now planned a combination of RIFLE and AKIN, and this definition seems likely to be globally accepted (Ronconi et al., 2009).

Table 1.1. Three different definitions of AKI with different stages.

	RIFLE			**AKIN**		**AKI**	
Stage	**Serum Creatinine criteria**	**MDRD eGFR Criteria**	**Anuria Criteria**	**Stage**	**Aerum Creatinin**	**Stage**	
Risk	>1.5×baseline over 7 days >2×baseline	>25%×decrease over 7 days		1	>1.5×baselineor >26mmol/l increase over 48 hours	1	> 26 mmol/l increase over 24 hours or >44 mmol/l over 48
Injury	over 7 days	>50%×decrease over 7 days		2	>2×baselineor over 48 hours	2	> 44 mmol/l increase over 24 hours or >88 mmol/l over 48 h
Failure	>3×baseline over 7 days	>75%×decrease over 7 days	, Anuria for>12h	3	> 3×baseline or >44 mmol/l increase if baseline >300 mmol/l over 48 hours or any RRT or anuria for> 12 h	3	> 88 mmol/l increase over 24 hours or >132 mmol/l over 48 h

Any degree of kidney injury, including mild and reversible AKI, can have significant implications for patient health and can lead to important clinical consequences including increased mortality (Oliver et al., 2004, Uchino et al., 2006). Indeed the published evidence indicates state that mild injury to the renal system begins quite earlier before the loss of function of kidney can be detected by using standard available tests (Safirstein et al., 1990, W., 2012).

1.5.2 Clinical significance of AKI

AKI is related with significant morbidity, mortality and common worldwide. AKI also has significant impacts on healthcare resource expenditure and is associated with an increased danger for progression to CKD particularly in patients who are critical ill (de Mendonça et al., 2000, Chertow et al., 2005, Uchino et al., 2005, Leung et al., 2012). A meta-analysis based study highlighted that the occurrence of AKI in adults

(21.6%) and children (33.7%) when KDIGO (the Kidney Disease Improving Global Outcome) definition was used (Susantitaphong et al., 2013).

Recently in 2015 Xiong et al in his twelve meta-analysis studies accounted 138,521 patients that have the AKI incidence in ICU (intensive care unit). Among them patients were detected with AKI based on the classification of RIFLE (50,054 patients) and classification of AKIN (50,521 patients). The AKI incidence ranges from 18.13%–66.67% and 24.14%–76.67% based on classifications of RIFLE and AKIN, respectively (Polyak and Hahn, 2006).

Earlier similar findings were reported by Bagshaw et al and Ostermann and Chang, and they state that the rates of AKI is considerably higher (upto 85%) in patients in within intensive care units (ICU) and about 15% required kidney replacement (Polyak and Hahn, 2006, Reya et al., 2001). Consistent with this, the UK Intensive Care National Audit Research Centre (ICNARC) in 2008 reported that about 10% ICU bed days is accounted by AKI (Kolhe Nv, 2008) and further AKI requirement of renal replacement therapy (Metnitz et al., 2002).

1.5.3 Etiology of AKI

Over the past decade the etiology of AKI has changed from earlier primary renal disease to complications of other systemic illness (Hui-Stickle et al., 2005, Symons et al., 2007, Askenazi, 2011). Even today AKI remains untreated in the absence of pharmacological treatments inspite of attempts that have able to successfully clinical staging, discovery of clinical based specific novel renal biomarkers, and ability to understand pathophysiology (Zuk and Bonventre, 2016). There is high prevalence of AKI due to shortage of skilled professionals in healthcare and under-utilization of detection tests and dialysis in lower- and middle-income countries. In addition to this

high burden on human and high number of under estimation of actual occurrence of AKI further deteriorates the problem. But in the developed countries, AKI seems too apparent in elderly patients in ICU. However in the less developed countries young adults and women belonging to lower or middle income class are at risk to succumb to death (Cerdá et al., 2008, Jha and Parameswaran, 2013).

AKI is commonly multifactorial, especially in adversely ill patients, resulting from conditions such as hypotension, sepsis, congenital heart disease, ischemic injury, nephrotoxins or malignancy (Hui-Stickle et al., 2005, Vachvanichsanong et al., 2006, Kale et al., 2003, Symons et al., 2007, Pannu and Nadim, 2008, Palmieri et al., 2009, Duzova et al., 2010, Schneider et al., 2010). The manifestations and clinical consequences of AKI are usually similar regardless of etiology.

AKI is traditionally pathologically classified by the location of the pathophysiology relative to the kidney; 'pre-renal' diseases alter the perfusion of the kidney, affecting oxygen delivery to the organ, 'intrinsic' diseases cause damage within the kidneys themselves, and obstructions of the urinary tract are considered 'post-renal' injury (Andreoli, 2009, Zappitelli and 2009, Gupta et al., 2002). Each of the conditions related to the development of AKI causes renal injury through different mechanisms.

Pre-renal AKI are consequences of renal hypo-perfusion due to low intravascular volume triggered by dehydration, fluid shifts outside the intravascular space or systemic vasodilation or low circulating volume from poor cardiac or, capillary leaks (Zappitelli and 2009). Although this is rare, pre-renal AKI can also arise due to renal artery stenosis or compression. A rapid replenishment of fluid volume to the kidney can restore renal function before acute tubular necrosis (ATN) occurs. ATN can also arise due to lack of oxygen/ischemia in renal tissue or toxic or vascular insults to the

kidney or through inflammatory mechanisms and leads to damage or destruction of the internal structures of the kidneys, particularly the tubules. This loss of tubular epithelia initiates the pathophysiological processes of ischemic renal injury restoration of intravascular volume not able to restore kidney function.

Any acute decrease in kidney function as an outcome of direct damage to renal tissue causes intrinsic form of AKI (Zappitelli and 2009), the most commonly observed AKI in the ICU as result of acute tubular necrosis. The post-renal AKI results from ureteral obstructions due to stones or blood clots.

In the paediatric ICU setting, this could rarely lead to AKI and is generally associated with tumors or mass effect, stones or blood clots and a common comorbid condition in critically ill children (Basu et al., 2011; Ricci et al., 2008). Unappreciated catheter obstructions in children with Foley catheters could also lead to the development of post-renal AKI.

1.5.4 Outcomes of AKI

In hospitalized patients AKI is associated with serious complications and is also related enhanced utilization of resource (Chertow et al., 2005, Lahoti et al., 2011, Parikh and Shaw, 2012), higher short- and long-term mortality (Chertow et al., 2005, Uchino et al., 2006, Bouzas-Mosquera A, 2009, Nin et al., 2010, Li et al., 2011, Mandelbaum et al., 2011, Wang et al., 2012b), and a higher risk for the CKD development (Lafrance et al., 2010, Chawla et al., 2011, Wu et al., 2011, Bucaloiu et al., 2012).

There were studies conducted to explain death of AKI patients. Based on RIFLE classification AKI patients were diagnosed (Basu et al., 2011) and out of these 13,423 patients suffered mortality in hospital. Likewise, 50,275 AKI patients were diagnosed

using AKIN classification and out of these 13,279 patients suffered mortality in hospital. So, the mortality in hospital based on classification of RIFLE and AKIN contributed to 0.27 and 0.26 respectively, however none of these classifications were statistically significant. In 110 studies that include 99 of Adults and 11 of children, used a KDIGO- equivalent definition and measured mortality as well as the pooled AKI-associated all-cause mortality rate that was 23.0% (95% CI, 22.1) (Polyak and Hahn, 2006).

1.5.5 Costs of AKI to healthcare systems

AKI is related to high individual morbidity and mortality causes heavy monetary burdens correlated with more length of stay in the hospital, requirement of more complex interferences and serious care services. Together with acute hospital admissions, critical care and renal replacement therapy (RRT), it is assessed that total costs in a year of AKI (Kerr et al., 2012) was 0.4 - 0.6% of the total UK NHS budget (range between £434 million and £620 million in 2009–2010) (Kerr M, 2012, Kerr et al., 2014). In addition, the long-standing cost of subsequent chronic kidney disease along with RRT, once end stage renal failure (ESRF) is achieved. Added to this is the long-term cost of following chronic kidney disease, together with renal replacement therapy if end stage renal failure (ESRF) is reached (Kerr et al., 2012). UK Intensive Care National Audit Research Centre (ICNARC) suggests from their data collection that nearly 10 percent of all ICU bed days for AKI (Kolhe Nv, 2008). AKI following RRT has been reported to account for 4.9% of all admissions to Intensive Care (Metnitz et al., 2002). The varying definitions of AKI stated in the literature leads to confusion while comparing these studies. However, it was over since 2004 when more specific terminology and most conventional definitions of AKI published (Sm, 2010, Cruz et al., 2010) that empowered healthcare professionals to define the disease more

precisely. This definition extended with a band of injury severity ranging from milder self-limiting forms to more advanced AKI possibly needs RRT. For these reasons the development of novel approaches to identify, treat, prevent and manage AKI is a clinical imperative.

1.5.6 Novel Biomarkers of AKI

A number of new peripheral biomarkers have been identified to improve the diagnosis of AKI that as certain initial stress responses of the kidney and appear in the plasma or urine before creatine levels in the serum become apparent (Devarajan, 2011). Neutrophil gelatinase-associated lipocalin (NGAL) is one of the most widely studied and validated initial stage biomarker in children for AKI. Increased NGAL measurements have also been correlated to the duration of stay in hospital and severity of AKI (Bennett et al., 2008, Dent et al., 2007, Goldstein, 2011, Krawczeski et al., 2011, Parikh and Shaw, 2012, Parikh et al., 2011). Using this approach, AKI become evident just one to two days before with corresponding increases in serum creatinine (Zappitelli et al., 2007, Wheeler et al., 2008, Zuk and Bonventre, 2016). Recently meta-analyses of NGAL studies in children and critically ill adults showed noteworthy associations between increasing NGAL levels with clinical outcomes that included mortality, duration of stay and the initiation of therapy for renal replacement and hence confirmed the clinical utility of this marker as an early indicator of AKI (Haase et al., 2009, Haase et al., 2011).

Serum cystatin C levels have also been highly correlated with AKI and used as a marker of disease progression after kidney transplantation (Gourishankar et al., 2008). Furthermore, kidney injury molecule-1(KIM-1), interleukin-18 (IL-18) and fatty acid binding-protein in liver are shown to be associated with renal ischemia

(Devarajan, 2008, Parikh et al., 2007). Recent reports have shown that the predictive ability of a biomarkers which have relatively low prognostic abilities to foresee worsening kidney function are enhanced when it combined with clinical markers (Koyner et al., 2010, Hall et al., 2011, Koyner et al., 2012, Arthur et al., 2014). Although, use of biomarkers is a rapidly evolving field, to date biomarkers have not been well integrated into clinical practice and therefore currently not a certain practical method to assess and diagnose acute kidney injury (Zuk and Bonventre, 2016).

1.6 Estimation of kidney function

In daily clinical management of kidney disorders, kidney products including serum-creatinine, urea and cystatin C, are the most common analyses conducted but for accurate assessment of kidney function GFR measurement corrected to the body weight is taken into account and this is conducted by measuring the clearance of a substance from plasma (plasma clearance). To evaluate renal function especially for medical investigation require large/ extensive sampling which include: creatinine, cystatin C, C-reactive protein, blood count, and estimated GFR, plasma-glucose, Sodium, potassium, sedimentation rate, liver function tests (ASAT, ALAT, LD, Bilirubin, GT, ALP), lipids (cholesterol, LDL, HDL, TG), albumin, parathyroid hormone levels, calcium and phosphate, urine-electrophoresis, uric acid, urea, urine dip sticks, diurnal U protein/albumin, Urine-electrophoresis, antibodies against basement membrane of glomerulus, titres of antibody for evaluation of antinuclear antibody, anti-neutrophil cytoplasmic antibody. Chronic kidney disease, is associated with severe kidney dysfunctionality and has several characteristic features like increased serum creatinine, anaemia, urea, urate and hyper-phosphataemia, acidosis.

hypercalcaemia. In addition to these sometimes it shows hypocalcaemia and hypo-albuminaemia and albuminuria conditions.

1.7 Renal cancer

It is estimated that ~3% of total malignant tumours in adults arise within the kidney. Cancers of the kidney are composed of a heterogeneous group of sporadic or hereditary cancers called renal cell carcinoma (RCC). According to Siegel et al, the frequency of kidney cancer is lower than for lung, breast, prostate and colorectal, but RCC is more lethal. It is projected that in United States there will around 62,700 new cases of cancers associated with kidney and renal pelvis in 2016 (Siegel et al., 2016).

1.7.1 Sporadic and hereditary renal cancer

Kidney cancer can arise both in sporadic and hereditary forms (Zbar, 2000, Phillips et al., 2001, Choyke, 2003). For example, sporadic RCC are typically unilateral and unifocal whereas hereditary renal tumours are often bilateral and multifocal. The age at diagnosis is significantly lower in hereditary kidney cancer (4th decade of life) as compared to sporadic renal tumours (6th-7^{th} decades). However, the sporadic forms of kidney neoplasms are much more common than the hereditary ones. Interestingly, sporadic renal cancers develop more frequently in men whereas the incidence of hereditary renal tumours is equal in men and women. One the main challenges in achieving a durable cure in patients with RCC is that the developing tumour often remains asymptomatic until it has metastasized. As outlined later, there are limited therapeutic options for metastatic RCC. For these reasons there is an urgent need for improved methods to diagnose individuals having higher chances of developing RCC and to identify patients harbouring RCC earlier in the disease process.

1.7.2 Hereditary renal cancer and the Von Hippel Lindau Syndrome

Hereditary forms of RCC have provided valuable insights into the molecular mechanisms contributing to RCC. Genetic and clinical studies of families with hereditary forms of kidney cancer revealed that renal carcinoma is comprises of pathologically distinct renal cancer types and is not a single disease. Based on their histo-pathology renal cancers are classified into several morphological subtypes (Kovacs et al., 1997b, Pavlovich and Schmidt, 2004, Linehan et al., 2007). Renal tumours can be identified as clear cell RCC (75% of RCC cases), papillary (10-15%), oncocytoma (5%) & chromophobe RCC (5%). Genetic diseases predisposing to the development of specific subtypes of kidney tumours and their associated contributory genes have been identified. There are four distinct types of hereditary renal cancer with their associated causative genes: von Hippel Lindau (VHL), hereditary papillary renal carcinoma (HPRC), hereditary leiomyomatosis renal cell cancer (HLRCC), as well as Birt-Hogg-Dubé (BHD) syndrome.

1.7.3 1.14 Hereditary renal cancer and the Von Hippel Lindau Syndrome

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1.7.3.1 VHL: Von Hippel-Lindau disease

Mutations of the *VHL* gene are implicated in the von Hippel-Lindau (VHL) syndrome (Linehan et al., 1995) which is linked to the development of tumours various organs including the kidneys in affected individuals, and development of clear cell renal cell carcinoma (Linehan et al., 1995). Up to 600 tumours per kidney can develop in VHL patients (Walther et al., 1995). VHL patients and in most sporadic clear cell RCC, mutation and loss of heterozygosity in the *VHL* locus were present that is supporting a tumour suppressor role for *VHL* (Gnarra et al., 1994, Herman et al., 1994, Lubensky et al., 1996, Shuin et al., 1994).

In normoxic condition, VHL protein, which belongs to E3 ubiquitin ligase complex, decreases the levels of the HIF-1α and HIF-2α transcription factors (Maxwell et al., 1999, Rosenberger et al., 2002, Miller et al., 2005) whereas absence of active *VHL* protein as observed in almost all ccRCC cases, leads to proteolysis of *HIF-1α* and *HIF-2α* in case of loss of absence of hypoxia. This cause constitutive increased expression level of HIF-1α and HIF-2α which is not depend on oxygen level (Krieg et al., 2000, Jaakkola et al., 2001, Prabhakar and Semenza, 2012). The loss of regulated *HIF* degradation is the consequences of loss of tumour suppressor function of *VHL* which in turn increases the expression of several proteins involved in angiogenesis like platelet-derived growth factor B chain (PDGF-B) and vascular endothelial growth factor (*VEGF*).

1.7.3.2 The VHL gene and germ line mutations

The *VHL* gene maps to chromosome 3p25 and the genetic linkage analysis have been performed in *VHL* families (Seizinger et al., 1988) which was later identified as the gene responsible for the *VHL* syndrome (Latif et al., 1993). The *VHL* gene comprises three exons and it encodes a 4.5kb-4.7kb mRNA (Kondo and Kaelin, 2001). It is conserved in rodents, flies and worms (Woodward et al., 2000). *VHL* expression is not restricted to the organ affected in the *VHL* disease but is widely distributed of both in human fetal and adult tissues (Latif et al., 1993, Iliopoulos et al., 1995, Kessler et al., 1995, Renbaum et al., 1996, Richards et al., 1996). Mutations of *VHL* are usually found in the *HIF* and elongin C-binding domains. Almost all VHL families identified germ line mutations of the *VHL* gene (Maher and Kaelin, 1997, Stolle et al., 1998) and *VHL* exist the characteristics of a tumour suppressor gene. More than 150 distinguished mutations have been reported and are widely distributed throughout the coding sequence, except for the first 50 codons where mutations are rarely observed (Zbar et al., 1996). Inactivation of *VHL* can occur by different pathways such as deletion (60-80%) or DNA methylation (20-25%) and genomic mutation (50-80%) (Kovacs et al., 1988, Velickovic et al., 2001, Kim and Kaelin, 2004, Kaelin, 2007). At the *VHL* locus the loss of heterozygosity (LOH) has been witnessed in the *VHL* patients with kidney cysts suggesting that cystogenesis would represent an early step in the development of RCC, although additional genetic events would be required for RCC to develop (Kim and Kaelin, 2004, Lubensky et al., 1996). Interestingly, when wild-type *pVHL is* reintroduced into RCC cell lines which are *VHL*-negative, it prevents formation of tumour in xenograft assays in nude mouse, supporting a tumour suppressor role for *phi* (Iliopoulos et al., 1995, Iliopoulos et al., 1996).

1.7.4 Histological/pathological classification of human kidney cancers.

In line with the classification of Heidelberg, histologic subtypes of RCC (Kovacs et al., 1997b) can be grouped into clear cell adenocarcinoma (most prevalent form of ccRCC), papillary RCC (pRCC), chromophobe RCC (chRCC), oncocytomas, collecting duct (Bellini's) RCC or unclassified RCC. Among infant tumours, the most commonly occurring renal cancer is Wilms' tumour (WT, nephroblastoma) which correspond to is ranked fourth for incidence cases of cancers in childhood (Pode-Shakked and Dekel, 2011). The classification of the subgroups is based on histological and genetic features and these represent the predominant subtypes although there are at least 16 different forms of RCC reported.

It has been observed that proximal tube is the site from where both clear cell and papillary renal cell carcinoma arise. It is possible to classify RCC based on the histological features or simply based on the genetic information. With the advancements in the identification of stage and histological diversity of RCC the World Health Organization (WHO) has transformation renal tumour classification. Furthermost common form of renal cancer is exhibited clear cell RCC that accounts for ~70% of adult renal neoplasms. ccRCC is followed by papillary variant (~15%), Chromophobe (~5%) and a rarer form of RCC i.e. collecting duct (~2%). Many studies have utilized analysis of classical cytogenetic, relative genomic hybridizations or other molecular genetic techniques to reveal several genetic modifications linked with various forms of RCC and also explain various steps intricate in the development and progression of tumour (Yuan et al., 1998, Tsui et al., 2000, Pantuck et al., 2001, Plebani and Piva, 2002, Kato et al., 2004, Sengupta et al., 2006).

The Fuhrman nuclear grading system has become extensively used (Fuhrman Sa, 1982). Its intra- and inter-observer reproducibility, however, has turned out to be rather low (Lanigan et al., 1994, Al-Aynati et al., 2003, Ficarra et al., 2005). However, it is debated how can we grade and how many grades are important (Bretheau et al., 1995, Ficarra et al., 2005). Flock and Kadesky introduced the first staging system for RCC in 1958 (Flocks Rh, 1958). Robson *et al*. modified the system in 1967 (Robson Cj, 1969). At present the most extensively used and generally recommended is the 2002 UICC TNM classification system (Table 1.1) (Greene et al., 2002, Salama Me, 2005). It takes into account the size of tumour, magnitude of local disease and occurrence of metastases when sorting patients for prognosis or selection of treatment. However, it is still uncertain whether this version of the classification is ideal for the prognostication of survival for patients suffering from RCC. It might be changed in the future. For many years tumours with the largest diameter less than 3cm were regarded as benign tumours/adenomas. However, in the last few years a new consensus suggests that all clear cell tumours should be considered carcinomas irrespective of size. It remains controversial where the cut-off point lies between the different subgroups in the present TNM classification system (See

Table 1.2 and 1.3).

Table 1.2: 2009 TNM classification system (Sobin LH, 2009)

2002 TNM classification and stage grouping for renal cell carcinoma

T	**Primary tumour**
Tax	No evidence of primary tumour
T0	Primary tumour cannot be assessed
T1a	Tumour 4.0 cm or less in greatest dimension, limited to the kidney
T1b	Tumour more than 4.0 but 7.0 cm or less in greatest dimension, limited to the kidney
T2	Tumour more than 7.0 cm in greatest dimension, limited to the kidney
T3a	Tumour invades adrenal gland or perinephric tissue but not beyond Gerota fascia
T3b	Tumour grossly extends into renal vein or *vena cava* below diaphragm
T3c	Tumour grossly extends into vena cava above diaphragm
T4	Tumour invades beyond Gerota's fascia
N	**Node**
Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastasis in a single regional lymph node
N2	Metastasis in more than one regional lymph node
M	**Distant Metastases**
Mx	Distant metastasis cannot be assessed
M0	No distant metastasis

Table 1.3: TNM stage grouping supplement 2012 (B.J. Wittekind, 2012)

Stage	T	N	M
I	T1	N0	M0
II	T2	N0	M0
III	T1	N1	M0
	T2	N1	M0
	T3	N0,N1	M0
IV	T4	N0,N1	M0
	Any T	N2	M0
	Any T	Any N	M1

1.7.4.1 Clear cell carcinoma: cellular origin (RPTE)

It has been estimated worldwide that every year about 3.38 million people develop kidney cancer, and more than 1 million people suffer mortality on account of this disease (Siegel et al., 2015, Torre et al., 2015). RCC is considered as the most common form of kidney cancer which exhibit high resistance to chemotherapy and can be distinguished from other types either by histopathological characteristics or by identification of specific gene mutations (Motzer et al., 2013). Nearly 75–85 % kidney cancers are accounted by ccRCC. It is believed to arise from tubular renal population especially thought to originate from the PT (Proximal tubule) epithelium of nephron (Bussolati et al., 2013). Regular networks of blood vessels which are of small and thin-walled represent a characteristic diagnostic feature of ccRCC. In addition, the tumour cells always have clear cytoplasm that is an outcome of resilient

intra-cytoplasmic deposition of glycogen, cholesterol, neutral lipids, and phospholipids (Siegel et al., 2015). ccRCC is a disease of altered cell metabolism (Linehan et al., 2010) and many genes known to involved in the development and/or progression of ccRCC are involved in sensing oxygen, iron, nutrients, and energy (Linehan and Ricketts, 2013). A well-known and highly accepted hallmark of cancer is altered metabolism (Hanahan and Weinberg, 2011a), and it is accepted that these metabolic alterations are largely effect tumour development as well as its progression (Jones and Thompson, 2009).

Families with VHL disease have a high incidence of ccRCC that occurs as a consequence of VHL inactivation. The genetic and molecular alteration attributable to the loss or deficiency of *VHL* results in an increased accumulation and activity of the hypoxia-inducible transcription factor (*HIF*). Subsequently that activates the expression of genes involved in angiogenesis (*VEGF, PDGF, SDF, CXCR4, TGFB1,* and *CTGF*), metabolism and glucose uptake (*HK2* and *PDK4*), pH control (*CAIX* and *CAXII*), metastasis/invasion (*MMP1, SDF, CXCR4,* and *MST1R*), proliferation and survival (*TGFB1*) (Allory et al., 2011, Little, 2006).

Alteration in *VHL* function contributes to increased expression of *HIF-1α* and *HIF-2α* (Little, 2006). One of the consequences of *HIF* over-expression is the increased expression of HIF target genes. One notable example is *NDUFA4L2* which is among the most highly expressed genes in almost all ccRCC samples compared to healthy kidney tissue and hypoxia induces *NDUFA4L2* expression via a mechanism involving *HIF-1α* (Safirstein, 1999). *NDUFA4L2* plays a role in altering cellular metabolism when *HIF-1α* is stabilized and active within a cell. Additionally, in tumours with wild-type *VHL*, mutations in *TCEB1* can be found. *TCEB1* encodes the Elongin C protein, which is a part of the *VHL*-complex responsible for degrading *HIF* under

normoxic conditions. These mutations are mutually exclusive from *VHL* mutations and chromosome 3p loss and occur in 1-5% of all ccRCC cases (Sato et al., 2013, Turajlic et al., 2015). The fact that the *HIF* pathway is affected also in wild-type *VHL* tumours, further stresses the importance of this pathway in ccRCC tumourigenesis. Most tumours tend to rely on aerobic glycolysis, in combination with lactic acid fermentation, instead of oxidative phosphorylation to meet their energy demand, a phenomenon called the Warburg effect (Ward and Thompson, 2012). By bypassing the tricarboxylic acid (TCA) cycle following glycolysis, and instead using lactate fermentation, significantly lower amounts of ATP is generated. However, this process also allows for the synthesis of membrane components and cellular building blocks that are important for cellular proliferation. Due to the loss of *VHL* and hence constitutive activation of the *HIF*, the Warburg phenomenon is especially prominent in ccRCC. Additionally, it has been shown that glucose metabolism is increased with higher grade in ccRCC and that the glutathione pathway is upregulated to inhibit potentially harmful reactive oxygen species (ROS) (Wettersten et al., 2015). In this study, an upregulation of tryptophan catabolism was also noted. Metabolites of the essential amino acid tryptophan has been associated with immune suppression in cancer (Chung and Gadupudi, 2011).

1.7.4.2 Papillary renal cancer

Another histological variant which is second most prevalent in renal cancer is papillary renal cell carcinoma (pRCC) which accounts for ten to twenty per cent of all renal cell cancers. It is not involve mutations in the VHL gene and exhibit different molecular characteristics compared to clear cell carcinoma (Twardowski et al., 2014). Based on histological and genetic characteristics pRCC can be classified into sub-types: type 1 and type 2. The characteristic feature of type 1 is single layered small

cell which consist of very less cytoplasm. Likewise type 2 is characterized by presence of large pseudostratified cells whose cytoplasm gets readily stained with eosin (Delahunt and Eble, 1997). Tumours belonging to type 2 exhibit more aggressive form of disease i.e. it is correlated with advanced stage of disease which has an enhanced frequency of necrosis (Pignot et al., 2007, Waldert et al., 2008, Klatte et al., 2009).

pRCC can present either as an inherited or sporadic malignancy, and both can be related to specific genetic mutations. Hereditary, and to a lesser extent, sporadic type 1 pRCC is linked with activating mutations of the proto oncogene *MET*, a tyrosine kinase receptor, which in these cases becomes constitutively active and thereby increase cellular proliferation. The familial pRCC type 2 is related to the loss of the tumour suppressor fumarate hydratase (FH), which causes hereditary leiomyoma renal cell carcinoma (HLRCC). In TCA cycle, FH converts fumarate to malate and loss of this enzyme thus lead to accumulation of fumarate. The increased levels of this metabolite cause *VHL* independent *HIF* stabilization by inhibiting the prolyl hydroxylases (PHD), which marks *HIFs* for *VHL* recognition. Surprisingly, type 2 pRCC is phenotypically and genetically very different from *VHL* mutated tumours, highlighting that *VHL* has more functions than simply targeting the *HIFs* for destruction. Additionally, it has been shown that the fumarate accumulation lead to *HIF* independent oncogenic effects by inhibiting Keap1 and thereby stabilizing the proto-oncogene *Nrf2*, a crucial transcription factor in the antioxidant response (Kinch et al., 2011). FH mutations have not been detected in sporadic type 2 pRCC, however, the hereditary and sporadic forms both display upregulation of antioxidant genes such as *AKR1B10*, which may explain their shared phenotype (Ooi et al., 2011). Other rare forms of hereditary pRCC can be attributed to mutations in *TSC1* and *TSC2*, which

are tumour suppressors and hamartin & tuberin encoding genes respectively. These mutations cause tuberous sclerosis complex, autosomal dominant disease that leads in patients to develop tumours in several body parts such as brain, lung, kidney and skin (Cheadle et al., 2000). Additionally, these patients also have an increased risk of developing ccRCC and chRCC.

1.7.4.3 Chromophobe renal cancer

Chromophobe renal cell carcinoma (CRCC) shows diverse histochemical, ultrastructural, and genetic features but it is one of the rare variant of renal carcinoma that accounts 4-5% approximately of all renal neoplasms (Crotty et al., 1995, Cheville et al., 2003). Chromophobe renal cell carcinomas (CRCC) have many promising diagnostic features. Most distinguish feature is the positive Hale colloidal iron cytoplasmic vesicles. In addition, the different extents of clear but poorly stained "chromophobe" cells along with hematoxylin and eosin stained eosinophilic cells are prominent characteristics. Expression of the proto-oncogene *KIT* as well as loss of chromosome 1, 2, 6, 10, 13, 17 and 21 is often seen in chRCC (Stec et al., 2009a). A study using data available on the cancer genome atlas (TCGA) showed, the most frequently mutated genes in chRCC are *TP53* (in 32% of cases) and in *PTEN* (in 9% of cases). The same study also showed that chRCC display recurrent genomic rearrangements affecting the TERT promoter and inducing elevated TERT expression (Davis et al., 2014). Hereditary chRCC is related with the loss of function mutations occur in the gene coding for follicular, *BHD*, causing Burt-Hogg-Dubé syndrome. Patients with BDH also develop lung cysts and oncocytomas, a benign form of RCC. Follicullin is advocated to play a part in the cells energy sensing mechanism via the AMPK and mTOR pathways (Linehan et al.) and in an animal model of BHD induced

kidney cancer, the mice displayed prolonged survival after treatment with the mTOR inhibitor rapamycin (Baba et al., 2008).

1.7.5 Diagnosis of RCC

No early warning sign is characteristic feature of RCC as localized small tumours hardly reflect any symptoms. Hence diagnosis is frequently delayed and disease can often only be recognized once it reaches in advance stage (Naudin ten Cate et al., 1995, Sinke et al., 1997). Hematuria accounts in 50 to 60 % of patients, abdominal pain in 40 % and a palpable mass in the flank or abdomen that present in 30 to 40 % of patients are some of the classical diagnostic symptoms related to renal cell carcinoma. Only 10% of patients are showing a combination of these three symptoms and that designate advance stage of disease. Several favourite sites such as lungs, bone, liver and brain accounts for approximately 25-30% of patients with metastatic disease at initial diagnosis. Cytogenetic analysis of human cancer cells generated or gathered large amount of information regarding incidences and abnormalities in chromosomes of malignant cells which have been used as vital tools in the diagnosis of new cancer cells. In addition, molecular characterization of specific gene involves in translocations lead to identification of several oncogenes, tumour suppressor genes and tissue specific genes. Altogether cytogenetic and molecular genetic analysis reveal essential information regarding oncogenesis, progression, diagnosis, and prognosis of cancer cell (van den Berg et al., 1993).

1.7.6 Treatments of RCC

Different types of procedures can be followed during surgery. For example in a partial nephrectomy, part of the kidney is removed while in nephrectomy, the entire kidney can be remove, it depends on how far the disease has spread. The more extensive

surgery like a radical nephrectomy may be followed to remove surrounding tissue, lymph nodes and adrenal gland. If both kidneys are removed, a dialysis or a transplant is necessary.

1.7.6.1 Radical and partial nephrectomy (RN)

Historically, the standard curative treatment for RCC has been radical nephrectomy (Robson Cj, 1969). More recently, surgical management of RCC has advanced through the development of laparoscopic approaches and nephron-sparing surgery (Duque et al., 1998, Uzzo and Novick, 2001). Currently nephron-sparing surgery is the regular treatment for small tumours in kidney. Laparoscopic radical nephrectomy is the new gold-standard procedure (Eskicorapci et al., 2007) and has to a large extent replaced the open approach which was the standard procedure between prior to 1994. More recently, robotic assisted laparoscopic surgery is become increasingly used.

1.7.6.2 Percutaneous cryoablation

Small renal tumours are increasingly being discovered over the last decade due to increased use of abdominal imaging performed for other purposes (Russo, 2000). Due to decreased morbidity, preservation of the renal function, and shorter hospital stay, ablative techniques that destroy tumour tissue have gained popularity. Among the ablative modalities, cryoablation is the best documented procedure for treating small renal cortical tumours (Mabjeesh et al., 2004). This minimally invasive procedure is based on freezing the tumour tissue by using nitrogen or argon. MRI or CT scans are used subsequently in order to assess the ablation. If the treatment is found to be inadequate a percutaneous biopsy, a standard resection, or retreatment with cryoablation can be performed.

1.7.6.3 Radiofrequency ablation

Some patients with a small RCC are unable to undergo nephron-sparing surgery because of comorbidities. In older patients with a small incidentally discovered tumour growing at a slow rate, that does not represent an immediate threat to the patients' life, watchful waiting and follow-up imaging can be appropriate (Birnbaum et al., 1990, Bosniak et al., 1995, Kassouf et al., 2004). However, CT-guided percutaneous radiofrequency ablation can be used to reliably eradicate small RCCs in patients who are unsuitable for surgery and desire a definitive treatment (Uzzo and Novick, 2001).It can be performed in a day-hospital for selected patients (Carrafiello et al., 2008).

1.7.6.4 Observation of renal masses

According to a recent study non-treated smaller renal masses detected in elderly and comorbid patients have a low growth rate of <1cm/yr in 85% of the cases, and100% 5-yr cancer specific survival (Beisland et al., 2009). These findings imply that this subgroup of patients that have high risk of postoperative morbidity and mortality could safely be selected for observation and not invasively treated.

1.7.6.5 Radiotherapy for metastases in RCC

Historically, RCC is resistant to radiotherapy. However, this treatment option can be apply to certain symptomatic patients with unresectable osseous lesions or brain (Fosså et al., 1982, Gez et al., 2002). Combined radiotherapy and the bisphosphonate, zoledronate, in patients with bone metastases from RCC can induce a higher response rate than radiotherapy alone (Russo, 2000). In individual cases whole brain irradiation, radio-surgery and/or a stereotactic radiotherapy can induce symptom palliation and improve survival (Kavolius et al., 1998, Andrews et al., 2004, Scorsetti et al., 2009).

1.7.6.6 Targeted molecular therapies

A defective *VHL* gene copy is the most common reason of inherited ccRCC. Furthermore, in most sporadic ccRCC diseased patients, the *VHL* gene is inactive. Hypoxia-inducible factor accumulation due to *VHL* inactivation, leads to production of several growth factors including *VEGF, TGFα* and *PDGF*, which promote neo-angiogenesis and get involves the development and progression of RCC. The *PDGF* and *VEGF* signalling pathways have been identified as targets for anti-cancer therapy. The targeting drugs, sorafenib, sunitinib, bevacizumab combined with INF-α, everolimus and temsirolimus have led to significant if modest improvements in progression-free survival and have been approved for metastatic RCC treatment (Yang et al., 2003, Patard et al., 2006, Patel et al., 2006, Hudes G and Global, 2007, Oudard et al., 2007).

1.7.7 Outcomes:

Improved initial detection of tumour and broadening of treatment possibilities that comprises tyrosine kinase inhibitors and mTOR inhibitors result into improved diagnosis for patients with RCC. However, these treatments rarely yield complete responses hence RCC is a tumour of unpredictable presentation and poor clinical outcome till date (Coppin et al., 2011). The challenges given, of prospectively identifying individuals at risk of sporadic RCC and the need for early diagnosis when treatments are most effective, there is an urgent cancer-specific survival need to develop new approaches to identify factors contributing to sporadic RCC risk in the genial population.

Hypothesis

In summary, both AKI and ccRCC are defined by aberrant activation of the hypoxic response. Collectively the evidences outlined above leads to the hypothesis that AKI can predispose to ccRCC. To test this hypothesis the following analyses were conducted:

RNA sequencing was used to analyze the presence of single nucleotide variants and aberrant splicing in the transcriptomes of wild type and *HIF-1α* over-expression model of ccRCC

RNA sequencing was used to compare the transcriptome of TRACK mice to human localized and metastatic ccRCC (TCGA) to identify genes required for metastases

RNA sequencing was used to compare transcriptomes from mouse model of ccRCC and porcine model of AKI. Identified differentiation expressed genes were validated by qRT-PCR.

Integration of genes common to mouse ccRCC, pig AKI in human ccRCC TCGA RNA sequencing data.

Chapter 2: Does over-expression of HIF-1α increase global transcriptional dysfunction in a mouse model of ccRCC

2.1 Introduction

In solid tumour there is a disparity between the oxygen consumed and the oxygen supplied to the tissue. This situation is termed hypoxia and provides a suitable microenvironment that is concomitant with its progression, metastasis, and resistance to radiation and chemotherapy (Haase et al., 2011, Brizel et al., 1996, Gourishankar et al., 2008, Devarajan, 2008, Jacobsen et al., 2000, Lubensky et al., 1996, Semenza, 2003, Vaupel, 2004, Chan and Giaccia, 2007, Sullivan and Graham, 2007, Liao and Johnson, 2007, Moeller et al., 2007, Shuin et al., 1994, Bertout et al., 2009, Prabhakar and Semenza, 2012, Seizinger et al., 1988). Therefore it considered as "Janus face" in tumour biology due to its association with controlled proliferation, differentiation, necrosis or apoptosis, along with the development of an aggressive phenotype (Durand, 1991, Giaccia, 1996, Riva et al., 1998, Haroon et al., 2000, Hockel and Vaupel, 2001, Semenza, 2002a, Semenza, 2002b, Koyner et al., 2012, Maxwell et al., 2001, Harris, 2002, Leo et al., 2004, Linehan et al., 2009, Vaupel and Mayer, 2007). There are a number of factors that are associated with anemia, perfusion or diffusion that can contribute to hypoxia (Vaupel et al., 2001, Vaupel et al., 2002, Hockel and Vaupel, 2001, Vaupel, 2004, Vaupel and Mayer, 2007, Bristow and Hill, 2008, Koyner et al., 2010, Siegel et al., 2016). The major mechanisms leads to hypoxia are (i) reduced supply of O_2 because of the unusual changes take place in the micro-vessel's structure as well as function, (ii) worsening of geometry meant for perfusion,(iii) there prevails a large distance between the cells of tumour and the

blood vessel supplying there, this decreases the diffusion and(iv) Anaemic condition may arise because during the tumour itself or because of treatment therapy (Vaupel et al., 2001, Vaupel et al., 2002, Vaupel and Harrison, 2004, Bristow and Hill, 2008).

Under hypoxic conditions the equilibrium of overall synthesis of proteins is disturbed which can lead to the arrest of cell cycle, cell differentiation, cell death and necrosis. The alterations in the genome and/or proteome because of hypoxia enables cells to conquer nutritive deprivation that help to get away from the hostile environment and favour unobstructed growth (Moulder and Rockwell, 1987, Durand, 1991, Giaccia, 1996, Riva et al., 1998, Haroon et al., 2000, Goda et al., 2003, Box and Demetrick, 2004, Koshiji et al., 2005, Choyke, 2003, Vaupel and Mayer, 2007, Vousden and Prives, 2009, Pires et al., 2010). When hypoxia is prolonged, and patient is treated with radiation or drugs it can disturb the distribution of cell cycle as well as the quiescent cell number (Vaupel and Mayer, 2007, Moeller et al., 2007, Keith et al., 2012). Hypoxia is also considered to be associated with the induction of apoptosis not only in normal cells but also in the neoplastic cells (Riva et al., 1998, Hockel and Vaupel, 2001, Vaupel and Harrison, 2004, Vaupel and Mayer, 2007, Azad et al., 2008). It is also well established that there is accumulation of p53 gene during the hypoxic condition and during this time apoptosis is induced by mechanism influenced by *HIF-1 α* (Amellem et al., 1997, An et al., 1998, Carmeliet et al., 1998, Koumenis et al., 2001, Linehan et al., 2009, Vaupel and Mayer, 2007, Sermeus and Michiels, 2011).

HIF-1α belongs to hypoxia inducible family (HIF), is a transcription factor and responsible for maintenance of homeostasis of oxygen or angiogenesis and is implicated in tumor development. Formation of newer blood vessels, growth of tumour, development & spread of tumour and metabolism of glucose are all regulated

HIF-1α. As stated earlier the *HIF-1α* as transcription factor regulates expression of key genes including *VEGF*: vascular endothelial growth factor, *EGFR*: epidermal growth factor receptors, *PDGF*: platelet derived growth factors, *IGF*: insulin-like growth factor, *GLUT-1*: glucose transporters, *CXCr*: chemokine receptors and CAIX & XII: Carbonic anhydrase (Koyner et al., 2012, Phillips et al., 2001, Maxwell et al., 1999, Zbar, 2000, Rosenberger et al., 2002, Bui et al., 2003, Miller et al., 2005, Pantuck et al., 2003, Grabmaier et al., 2004, Tang et al., 2004, Chen et al., 2005, Pages and Pouyssegur, 2005, Kim et al., 2005, Kon-no et al., 2006, Koukourakis et al., 2003, Pore et al., 2006, Klatte et al., 2007, Ljungkvist et al., 2007, Sandlund et al., 2007, Vaupel and Mayer, 2007, Lidgren et al., 2005, Tzao et al., 2008, Lee et al., 2009, Li and Kaelin, 2011, Siegel et al., 2016, Kroeger et al., 2014). *HIF-1α* also known to have regulatory function in cell cycle and cell death (Schmid et al., 2004, Linehan et al., 2009, Xu et al., 2010, Gudas et al., 2014, Razorenova et al., 2014). Although it acts as central regulator of tumoural pathophysiology, there is some controversy regarding *HIF-1α* role in RCC (Gudas et al., 2014, Olenchock et al., 2016).

As discussed in the introduction, RCC may be classified into three types such as papillary (pRCC), chromophobe (chRCC) and clear cell (cRCC). More vascularization is seen in cRCC compared to other forms (Kovacs et al., 1997a, Jaakkola et al., 2001). High vascularization in cRCCs causes modification of the suppressor gene (*VHL*: von Hippel-Lindau gene) subsequently the products of the modified gene fail to degrade *HIF-1α* as process was influenced by oxygen. Significant amount of research support the findings that anoxic or hypoxic conditions in tumor and HIF-system are together have a cumulative positive effect on the tumor cells and help in the transformation into phenotypes of progressive malignancy

(Hockel and Vaupel, 2001, Semenza, 2002a, Semenza, 2002b, Harris, 2002, Leo et al., 2004).

2.1.1 Genome Instability:

2.1.1.1 Hypoxia mediated genetic instability

Genetic instability is observed as a hallmark feature of most human cancers (Hanahan and Weinberg, 2000, Delahunt and Eble, 1997, Lindgren et al., 2011, Nissenson, 1998, Pikor et al., 2013, Macheret and Halazonetis, 2015).There can be instability in the genetic makeup that may induce alterations in the genes as a result of point mutations, alterations in number of chromosomes or chromosomal translocations and result in fusion transcripts. The genetic changes both at nucleotide level (due to base substitutions and deletions or insertions of a few nucleotides) and at the chromosomal level (involving chromosome loss or gain, translocations, and rearrangements) causes instability (Venkatachalam et al., 2010). Furthermore, many human tumours are genetically heterogeneous as a consequence of a mutator-phenotype. It is now a known fact that genetic instability is correlated to progression of tumour. New studies suggest that the microenvironment of the tumour itself has the capability to lead to genetic instability in the tumour cells (Li et al., 2013, Vaidya et al., 2008, Lam et al., 2005). The conditions in this microenvironment are hostile and can contribute to mutations and DNA damage (both oxidative base damage and breaks in DNA strand). The lesions in the DNA may contribute to the loss of integrity of genome (van den Berg et al., 1993, Naudin ten Cate et al., 1995). Studies in the recent past advocate that the instability in the microenvironment of the tumour can also arise because of loss of regulatory function in DNA repair pathways (Haase et al., 2009, Vaupel and Harrison, 2004, Kellum and Chawla, 2016).

Genetic instability and mutated phenotypes can be influenced by the hypoxia, which can lead to apoptosis resistance and inability to repair DNA. Hypoxia could enhance the mutation rates and alter chromatin functions. This explains in part how the tumour cells adapted to the lower O_2 levels keep on proliferating in compromised DNA repair (Coca et al., 2012).Critical hypoxia can enhance production of *HIF-1α*. This could up-regulate the expression of other genes such as *VEGF* and *LOX* (lysyl oxidase) that are responsible for angiogenesis and metastasis. Extended exposure of cells to hypoxia can lead to genetic instability as a result of decrease in DNA repair proteins. As a result there is improper repair of DNA in proliferating cells and this contributes to enhanced rate of mutation (Bristow and Hill, 2008). The repair of double stranded breaks (DSB) in DNA is known to be inhibited to by hypoxia and hypoxia may promote instability of chromosomes in many ways (Haase et al., 2011, Giraud et al., 2011, Sinke et al., 1997). In the chromosomal DNA there are specific fragile sites which are vulnerable to breaks and rearrangements during replication process and these tend to get induced in conditions of low oxygen (Giraud et al., 2011, Varrier et al., 2015).When DNA is amplified from the unrepaired DSB it is observed to resemble with the cells in hypoxic condition (Malek and Nematbakhsh, 2015, Haase et al., 2011, Wang and Lin, 2014, Van den Berg et al., 1994, Xu et al., 2015). In addition to this, it has been observed that in the human lymphocytes exposed to hypoxia the rate of sister chromatid exchange that is partially controlled by homologous recombination repair also increases (Oh and Park, 2015, Kovacs et al., 1997b). Likewise it has been observed that human fibroblasts exposed to continued low oxygen after induction of DNA damage exhibited enhanced chromosomal abnormalities such as breaks in chromatids/chromosomes, tetrameric unions, ring chromosomes, reciprocal translocations and double minutes (Tang et al., 2009).

Hypoxia can also contribute to (i) methylation or de-acetylation of histone proteins or *H2AX* phosphorylation and (iii) alteration in the condensation states of chromatin (Sinke et al., 1997).

2.1.1.2 Hypoxia and DNA damage response

Daily significant amount of DNA damage and mutagenesis arise as a result of (i) various reactive endogenously metabolites, (ii) errors in replication and (iii) spontaneous decay (Zhang et al., 2012). There are various check points in the cell cycle regulation that prevent carcinogenic proliferation of genetically damaged cells. However, recent experimental findings suggest that both hypoxia and anoxia can disrupt these checkpoints and thereby interfere with the repair of damaged DNA. G1 and S (interphase) phases of DNA replication can be arrested as a result of anoxia. It has been shown that activation of DDR (DNA damage response), apical kinases, ataxia telangiectasia mutations such as *ATM* and *ATR* (ataxia telangiectasia and Rad3 related protein) and phosphorylation of targeted downstream genes (*p53, Chk1* and *Chk2*) can be induced under stressful replication as a result of hypoxia; even when the DNA in not damaged (Blank and Shoenfeld, 2008, Brugarolas, 2014, Lu et al., 2013). However, when these cells were subjected to re-oxygenation rapid DNA damage was observed and cells were arrested at G2/M phase as a result of orthodox Chk2-dependent DDR response (Gardner et al., 2014, Lu, 2014). This could lead to activation of *ATM* and *ATR* and Rad3-related protein kinases that mediate cell cycle regulation at checkpoints. The cell cycle is arrested in order to look for repair of DNA damaged as a result of ROS (reactive oxygen species). It has been suggested that early tumourigenesis is associated with replicative stress followed by induction of DDR which try and act as a barrier to prevent progression of tumour (Hernandez-Yanez et al., 2012, Jones et al., 2005).

2.1.1.3 Hypoxia –mediated inhibition of DNA repair

Many factors can potentially alter the DNA repair and fate of DNA once the DNA is damaged as result of endogenous or exogenous factors in the solid tumours under low oxygen conditions. It is suggested that hypoxia or anoxia may not always be responsible for damage of DNA during S-phase of cell cycle, however when these cells are re-oxygenated, ROS such as single-stranded & double-stranded DNA binding proteins (DNA SSBs and DNA DSBs respectively) are generated which are responsible for damage of DNA which in turn activate the *CHK2* dependent G2 checkpoint. Continued exposure of cells to hypoxia can lead to the adaptations in the cell and cell and cell may not display activation of G1 or S checkpoint (Gardner et al., 2014, Lu, 2014, Linehan et al., 2007, Bristow and Hill, 2008, Siegel et al., 2016). As a result there would be no corrections in the altered DNA in course of replications, and this could potentially lead to accumulation of replication errors or breaks in DNA (Linehan et al., 2007). Repair of Human DNA exhibiting double stranded breaks can be repaired by pathways involving (i) HR: homologous recombination as well as (ii) NHEJ: non-homologous end joining (Fu et al., 2015, Fu et al., 2011, Gao et al., 2013). These two pathways differ with respect to protein components, specificity of cell cycle and commitment of DNA repair. If the normoxic cells exhibit inhibition of HR and NHEJ repair pathways, it is linked with enhanced genetic variability and carcinogenesis (Fu et al., 2013, Cerami et al., 2012, McLaren et al., 2016, Minton et al., 2015, Quinlan and Hall, 2010, Wang et al., 2010a). Thus to maintain the integrity of the genome, retention of error free HR is important specifically under normoxic and hypoxic condition as it alter the sensitivity of agents responsible for DNA (Lam et al., 2005).

2.1.2 Role of HIF-1alpha

2.1.2.1 Oxygen dependent regulation

Severe hypoxia can lead to replication arrest which is not dependent on the S-phase checkpoint, DDR: DNA damage response and transformation grade. However, the seized hypoxic cells (due to fluctuating tumour oxygen tensions) can endure rapid reperfusion and re-oxygenation leading to reoxygenation persuaded DNA damage. Reoxygenation-induced replication restart is inhibited in chronically exposed cells and is associated with repression of numerous replication factors, including *E2F*-dependent repression of *MCM6* and the loss of phosphorylation and correct localisation of RPA which, in turn leads to a decrease in the hypoxia-induced DNA damage response (Pires et al., 2010).

2.1.2.2 Oxygen independent regulation

In contrast, re-oxygenation-induced replication can occur after acute exposures to hypoxia. This is however associated with DNA damage on re-oxygenation and repair of DNA is incomplete. Re-oxygenation of cells following acute hypoxia leads affect *p53* gene which induces cell death. These studies indicate that cells where functional *p53* is absence, re-oxygenation after acute (but not chronic) exposure to lower oxygen, can contribute to increased genomic instability and potentially tumourigenesis (Pires et al., 2010).

2.1.2.3 HIF-1α expression in human cancers

Metabolic alterations are a distinguishing characteristic of ccRCC. It has been observed that HIF1α is important for the altered metabolism in tumour in TRACK model of ccRCC. This which what is also observed one human ccRCC (Gudas et al., 2014, Bellomo et al., 2004, Fu et al., 2011, Fu et al., 2015, Minton et al., 2015,

Pavlovich and Schmidt, 2004). In people development of ccRCC is associated with transcription factor (*HIF-1α)*. Over a decade of research has highlighted the role of *VHL* target genes (*HIF-1α* and *HIF-2α*) in renal cancer (Keith et al., 2012). Most of these suggest that over expression of *HIF-1α* is critically important for ccRCC tumour genesis.

The functional loss of *VHL* tumour suppressor gene and the resulting loss of regulated HIF degradation in ccRCC cells results in the increased expression of several proteins transcriptionally activated by *HIF-1α* that are involved in angiogenesis, such *PDGF-B* and *VEGF*. Normally, the *HIF-1α* gene is only stimulated in the low oxygen conditions and is responsible for the adaptation of the tumour to hypoxic conditions. The amplified expression of *VEGF* in ccRCCs explains the vascularity of these tumours and directly led to the expansion of a variety of treatments that can precisely aim the VEGF pathway. *HIF-1α* is known to influence phenotype change in tumour in a major subset of ccRCCs and that therapeutic strategies aimed at targeting *HIF-1α* and the genes it affects should continue to be explored.

Genes transcriptionally activated by *HIF-1α* include *NDUFA4L2* (Pavlovich and Schmidt, 2004). NADH Dehydrogenase (Ubiquinone) 1α subcomplex 4-like 2 (*NDUFA4L2*) is a novel molecular marker for clear cell renal cell carcinoma (ccRCC) treatment. Data obtained from several sets of microarray from Oncomine database and RNA sequencing data from The TCGA (Cancer Genome Atlas) revealed that *NDUFA4L2* is highly expressed in the ccRCC samples when compared with the healthy kidney (Lenburg et al., 2003, Jones et al., 2005, Gumz et al., 2007, Yusenko et al., 2009). Similarly *NDUFA4L2* expression also increased more than fifty fold in TRACK kidneys. It is also seen that the patients exhibiting highest *NDUFA4L2* expression has poorest survival outcomes (Pavlovich and Schmidt, 2004).

NDUFA4L2 is known to lower consumption of mitochondrial oxygen and activity of mitochondrial Complex-I under hypoxic condition, thus shifting from aerobic mitochondrial respiration to anaerobic glycolysis and reduced production of ROS. *NDUFA4L2* expression is stimulated by hypoxia through a mechanism involving *HIF-1α* rather than *HIF-2α.*

2.1.3 Hypothesis

Given the established role of *HIF-1α* in mediating the hypoxic response in ccRCC and the role of hypoxia in genome instability, the effect of *HIF-1α* over-expression on DNA mutations in the transcriptome and transcriptional fusions was tested using next generation RNA sequencing of specimens from *HIF-1α* overexpressing TRACK mice.

2.2 Material and methods

2.2.1 cDNA library preparation and sequencing:

Total RNA from control (n=3) and HIF-1α-over-expression (n=3) transgenic mice were submitted to the genomics facilities at Weill Cornell Medicine (Fu et al., 2011) for single-end Illumina HiSeq RNA sequencing.

2.2.2 Raw read filtering and read mapping:

Raw fastq sequences obtained from NCBI-GEO (Fu et al., 2011) and were analyzed using a local Linux cluster. The sequence data in fastq.gz files were aligned using TopHat2 and BWA to Illumina iGenome ENSEMBL annotated mouse (GRCm38/mm10) reference sequences and annotations (Langmead et al., 2009, Trapnell et al., 2009). SAMtools was used to convert BWA-generated SAM files to BAM format. The resultant TopHat2 and BWA generated BAM alignment files (for each condition) with transcript annotation file were provided as input to Cufflinks/cuffdiff (Trapnell et al., 2010) to quantify transcripts from RNA-Seq reads. These reads had been aligned to the genome and counted the number of reads that mapped to the genomic location of that transcript hence able to predict the relative abundances of each transcript. Abundances were stated in RPKM which represented the expected reads per kilo base of transcript per million fragments sequenced (Trapnell et al., 2010). Both TopHat and BWA alignments were used in SNV analysis. The all possible logical relationship between Control and TRACK mice SNVs obtained from TopHat and BWA alignments were analysed through Venn-diagram. Further Venn analysis was also done to identify SNVs unique to the TRACK mice.

Venn Analysis:

Gene lists using common gene identifiers were generated using the SNV pipeline described earlier. The overlap of genes affected by SNVs identified by Tophat and BWA pipelines were determined using Venny software available at http://bioinfogp.cnb.csic.es/tools/venny/.

First SNVs common to both control groups and SNVs common to both TRACKs of BWA and TopHat were identified and pasted one element per row on online Venny software in two separated lists named Control BWA_TopHat and TRACK BWA_TopHat. Further results were observed and figure were viewed and saved by right click.

2.2.3 Differentially expressed gene analysis:

Transcripts assembled by cufflinks were used by Cuffdiff to compared gene expression between two samples (Normal and TRACK); identify differential isoform and differential promoter usage. The reads for each biological replicates were mapped independently. In the comparisons values were considered to exhibit changed expression if the $p < 0.05$ and there was at least two fold change in expression in Cuffdiff output. Further Differential gene list was analyzed for gene annotation (GO Analysis) and matched with The Cancer Genome Atlas (TCGA) research network ccRCC.

2.2.4 Detection of SNV and specific point mutations in tumour:

To detect SNV (single nucleotide variants) in the HIF-1α-over expressing TRACK mice and, quality processed-fastq files were aligned to the iGenomes ENSEMBL annotated mm10 genome using BWA (Li and Durbin, 2009) and TopHat2. To analyze

SNV (Li and Durbin) and specific point mutation in aligned transcriptomics data the SAMtools package that involved SAMtools and BCFtools. The SAMtool calculates the genotype likelihood L (g) that considers error dependencies whereas BCFtools realizes variants and applies various statistics. To compare SNV calls generated the SAMtool assembled, each sample was aligned using both duplicate read methods to the reference genome (mouse ENSEMBL) specifically when aligning reads to the reference genome compared to a transcriptome-only approach

To compare SNV calls generated the SAMtool assembled when aligning reads to the reference genome compared to a transcriptome-only approach and therefore. The analysis of SNV was done for all three mice. Later the ENSEMBL-Variant Effect Predictor (VEP) (McLaren et al., 2016) was used to predict or identify new variants. Also list of genes present in TRACK mice was checked for their implication in ccRCC using cBio portal of the TCGA provisional dataset (Cerami et al., 2012, Gao et al., 2013).

2.2.5 Detection of alternative splicing:

The MapSplice tool was used to identify novel transcripts or fusions (Wang et al., 2010b) specific to the *HIF-1α*-over-expressing TRACK mice. The novel exon junctions generated for each sample by MapSplice were converted to BED file format and then concatenated to yield control and TRACK junctions. Identified splicing novel transcript-fusion events were considered where >10 RPKM supported the junction (Quinlan and Hall, 2010). The junctions from each MapSplice output were converted to BED file and then concatenated to yield control and TRACK junctions. Bedtools intersect (-v option) was completed for TRACK (-a file) versus control (-b

file) to yield TRACK unique junctions in BED format. Splicing was analyzed using the IGV-sashimi plot function.

2.2.6 Detection of canonical and aberrant alternative splicing events

The MapSplice tool was used to identify novel transcripts or fusions (Wang et al., 2010b) specific to the HIF-1α-over-expressing TRACK mice. The novel exon junctions generated for each sample by MapSplice were converted to BED file format and then concatenated to yield control and TRACK junctions. Identified splicing novel transcript-fusion events were considered where >10 RPKM supported the junction (Quinlan and Hall, 2010). Splicing was analyzed using the IGV-sashimi plot function.

2.3 Results

SNVs were identified using both TopHat2 and BWA generated alignments in both control (wild type) and TRACK mice relative to MM10 reference genome. Venn analysis was then completed to identify SNVs unique to the TRACK mice (Figure 2.1). Comparison of control and TRACK mice genes single nucleotide non-synonymous coding variants (SNVs) contained within RNA sequence data. Only variants identified by both TopHat and BWA alignments that alter coding sequences are included in analysis.

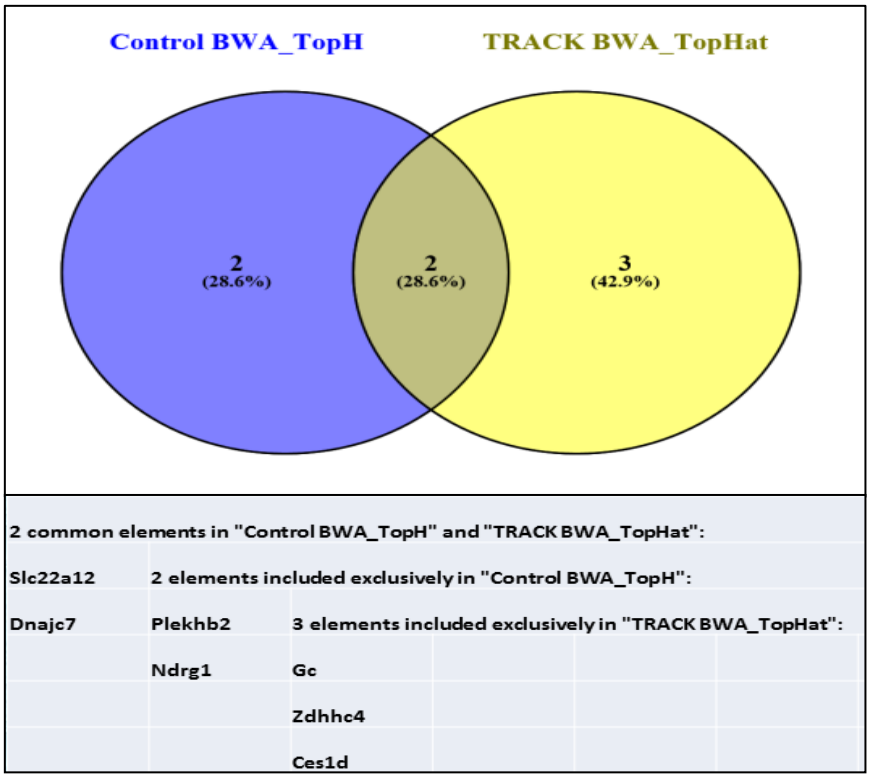


Figure 2.1: Comparison of single nucleotide non-synonymous coding variants identified in control and TRACK mice genes SNVs contained within RNA sequence data. Only variants identified by both TopHat and BWA alignments are indicated.

Venn diagram identified two coding variants unique to control, two present in both Control and TRACK mice, and three unique coding variants exclusively in TRACK mouse. These coding variant accounted 28.6% in control, 28.6% common to both control and TRACK and high 42.9% in TRACK of total SNVs analysed (Figure 2.2). Exclusive TRACK coding variants were *GC, ZDHHC4* and *CES1D* genes. The variant effect predictor showed high number of variants and number of affected genes are in TRACK in comparison to control.

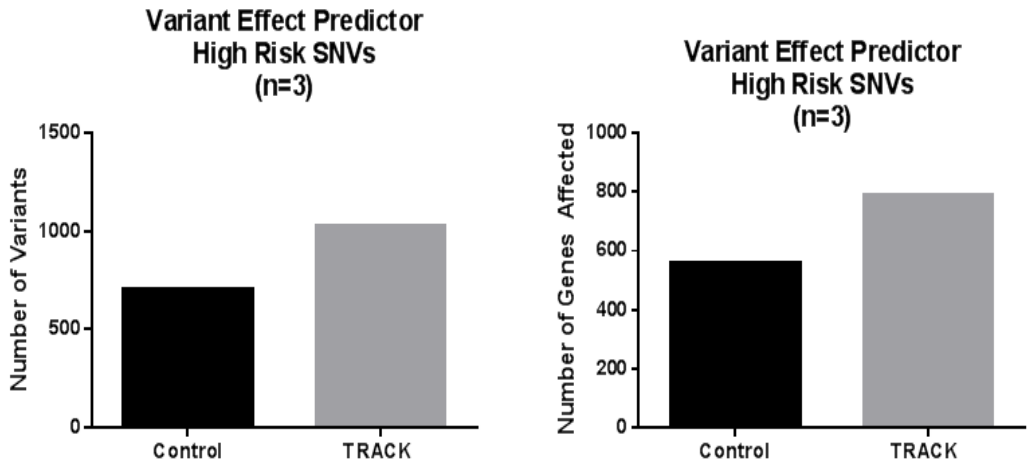


Figure 2.2: Histogram illustrating comparison of number of variants and number of genes affected in control and TRACK predict by Variant Effect Predictor (VEP). The track mice showing both high number of SNVs and number of gene affected in comparison to control

The prevalence of mutations in these genes was checked in ccRCC patients using renal cancer cBio mutations using TCGA/KIRC Genome dataset. All the three genes showed mutations when compared to all and renal cancers (Fig. 2.3). Further, these genes were checked for mutation in human ccRCC using TCGA/KIRC Genome dataset and out of *GC* and *ZDHHC4 and CES.* Only *CES3* gene showed mutations (Fig. 2.4).

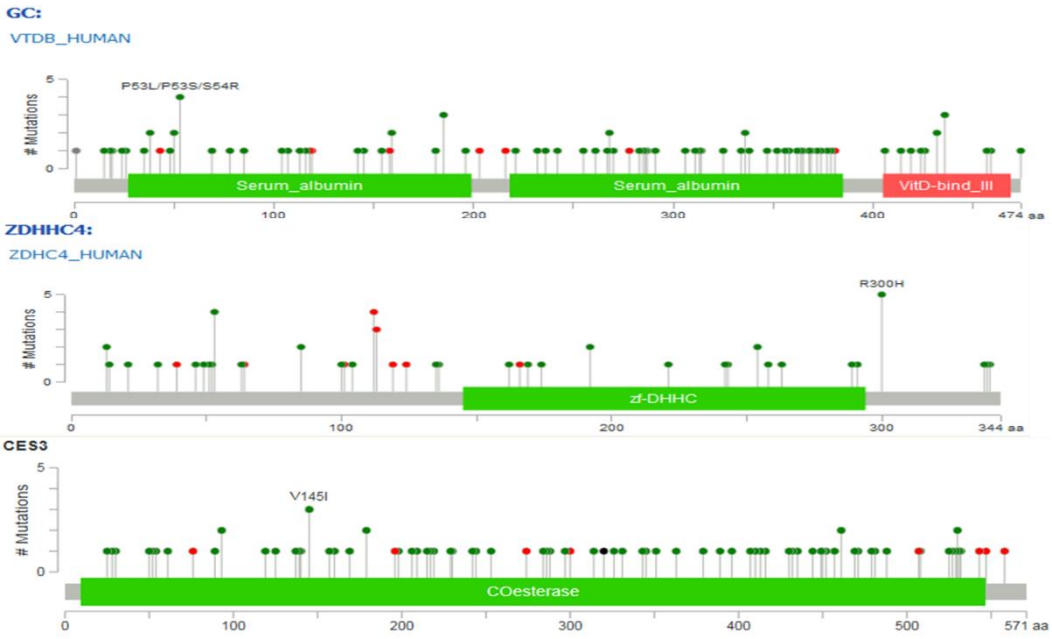


Figure 2.3: Schematic representation of SNVs identified in the *GC*, *ZDHHC4* and *CES3* gene in all cancers reported in cBIO.



Figure 2.3: cBio Analysis using TCGA/KIRC Genome dataset showing *CES3/Ces1d* is mutated human ccRCC whereas no mutations in *ZDHHC4* and *GC* gene is observed in ccRCC patients.

Ces1d (known as *CES3* in mice) was the only one unique coding variants identified by both TopHat and BWA alignment that alter coding sequences. Different genetic alterations of the *Ces1d* were checked in oncoprint of cBioPortal using TCGA/KIRC Genome dataset analyzing 413 patients/samples and illustrated amplification in 5% of human ccRCC, but not mutation or deletion as observed for the *CES3* gene (Fig. 2.5).

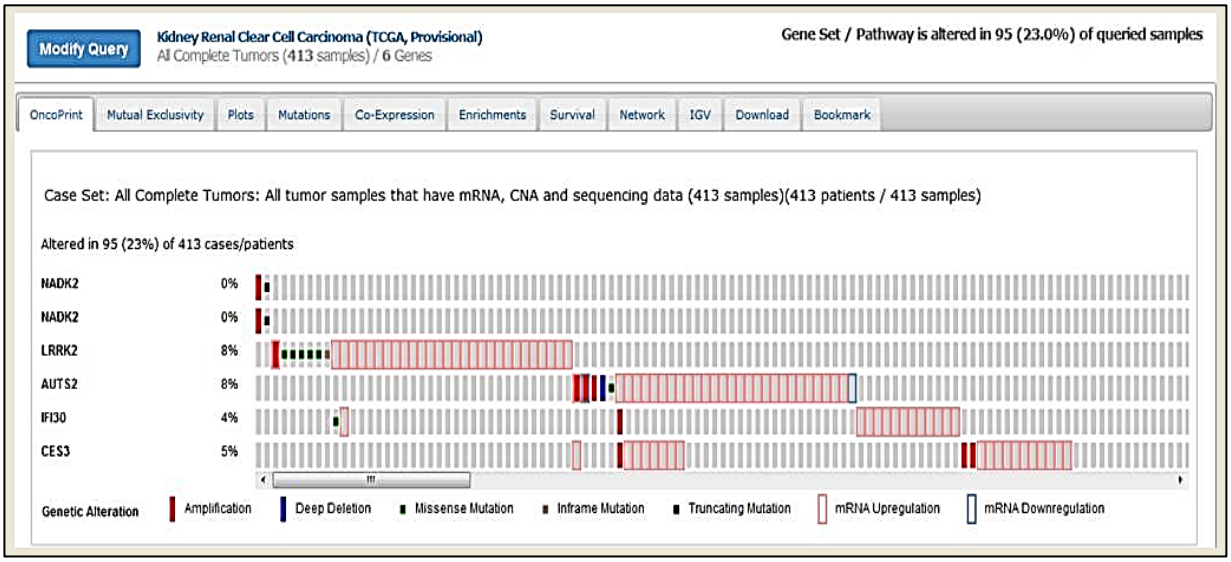


Figure 2.4: Oncoprint depict genetic alterations in *CES3* gene analyzed on cBio using TCGA/KIRC Genome dataset (N=413). The *CES3/Ces1d* is showing amplification in 5% of human ccRCC. No other alterations are shown.

Additionally, *CES3* gene alteration frequency was checked for all cancer types and showed amplification of gene in most of the cancer whereas some of the cancers depicted mutation and deletion (Fig. 2.6). When analysis was restricted to different subset of RCC the *CES3* gene showed amplification in ccRCC and mutation in papillary renal cell carcinoma (pRCC) whereas no alterations were noticed in the *CES3* gene in chromophobe (chRCC) and non-clear cell carcinoma (nccRCC) (Fig. 2.7).

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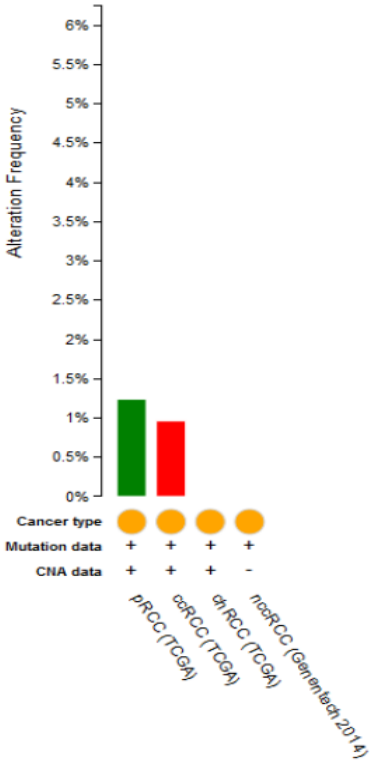


Figure 2.5: Histogram depicts different alteration in gene CES3 in different subset of renal cell carcinoma (RCC) on cBIO using TCGA/Genen tech 2014 KIRC Genome dataset. The *CES3* gene shows mutation in papillary renal cell carcinoma (pRCC) and amplification in clear cell renal cell carcinoma (ccRCC). There is no alteration in chromophobe renal cell carcinoma (chRCC) and non-clear cell renal cell

Further, Cuffdiff and MapSplice splicing analysis of the TRACK dataset was used to test for alternative and aberrant splicing events affecting genes implicated in ccRCC including *HIF-1α, HIF-2α, VHL, NDUFA4L2* and *VEGFA* along with newly identified coding variant *CES3/Ces1d* identified in TRACK. No aberrant novel splicing variants in the transcripts of *HIF-1α, HIF-2α, VHL, NDUFA4L2* and *VEGFA* were identified. Only *Ces1d* transcript showed splicing SNVs (Fig. 2.9 & 2.10).

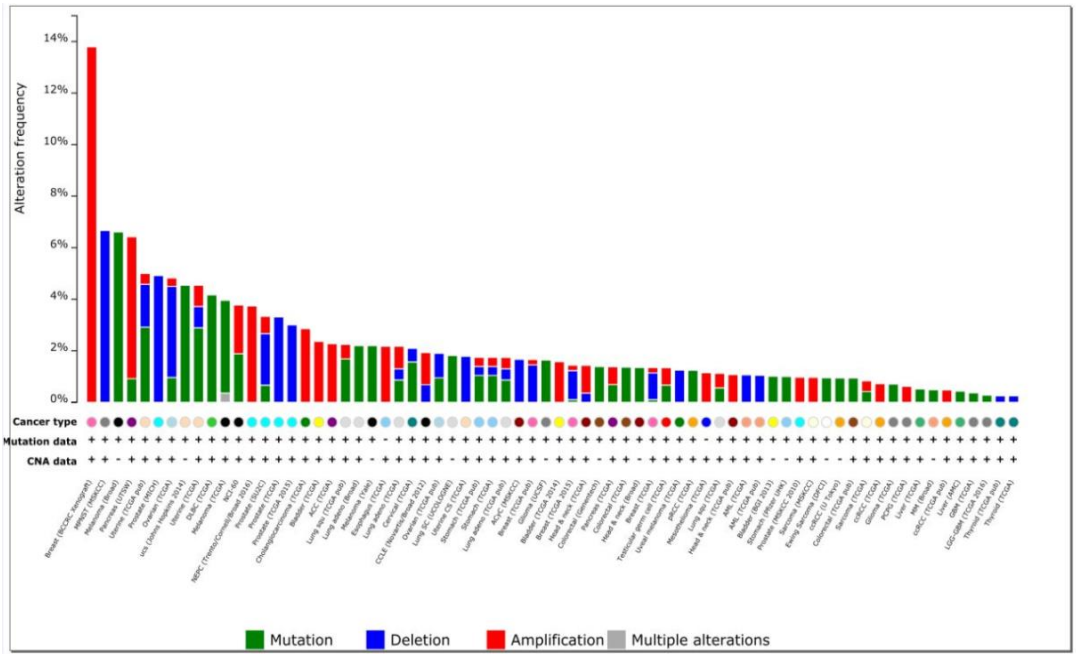


Figure 2.6: Histogram depicts different type of alterations in *CES3* gene in overall cancer analyzed on cBIO using different human gene database. Several other cancer showing amplification (Red color) of *CES3* gene.

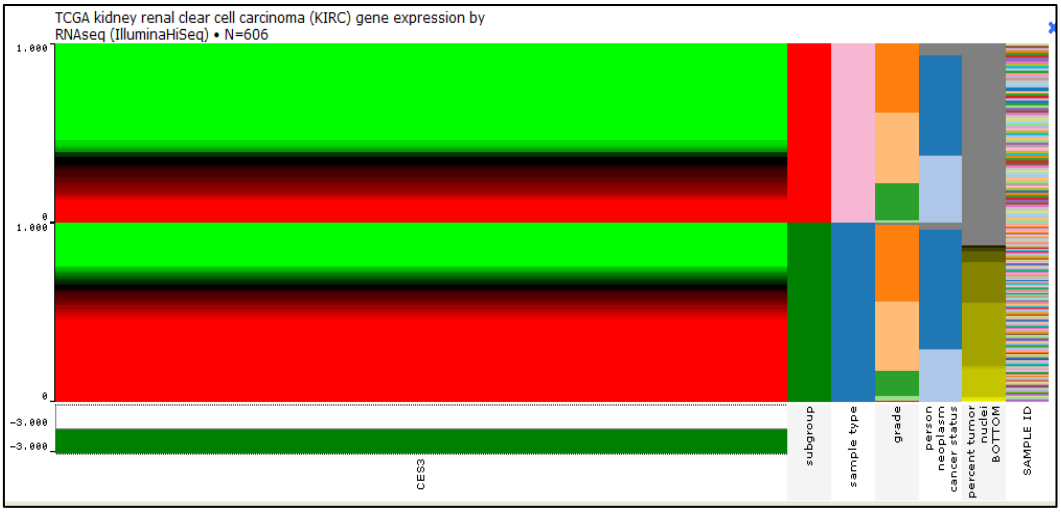
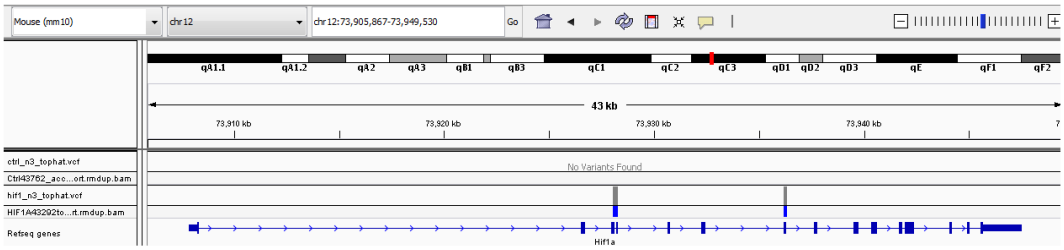


Figure 2.6: Gene expression level of *CES3* gene in tumour (Green) and solid tissue (normal, Red) investigated using TCGA/KIRC human genome dataset on Cancer Genome Browser. *CES3/Ces1d* is significantly higher in tumour relative to non-tumour specimens in ccRCC

HIF-1α



HIF-2α



VHL SNVs

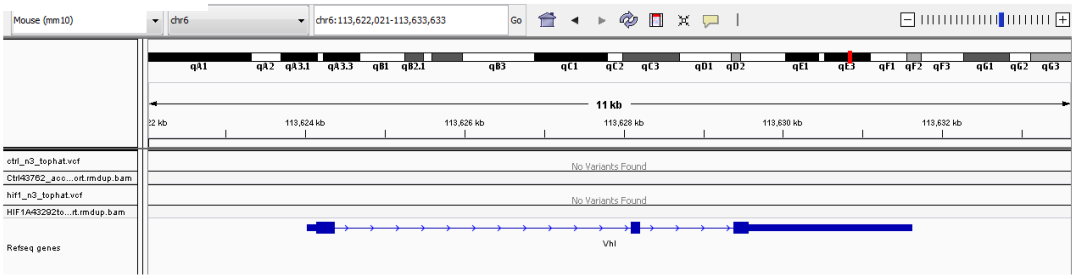
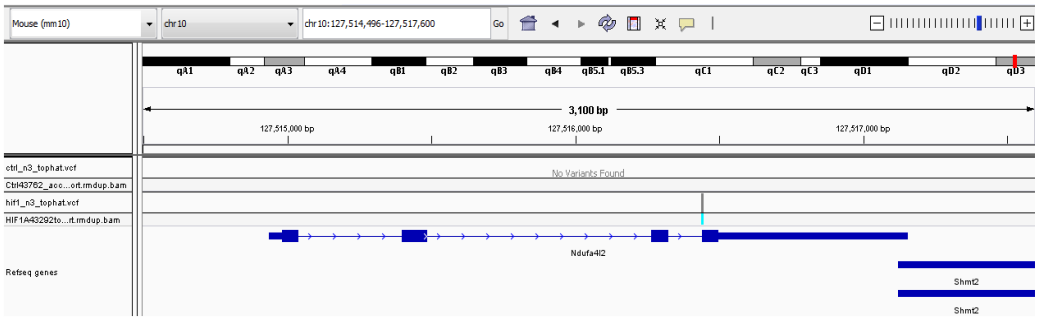


Figure 2.7: Splicing variants shown by SNVs affecting the known ccRCC gene and SNV identified in TRACK mice on Integrated Genome Viewer. *HIF-1α, HIF-2α* and *VHL* are not showing splicing variants.

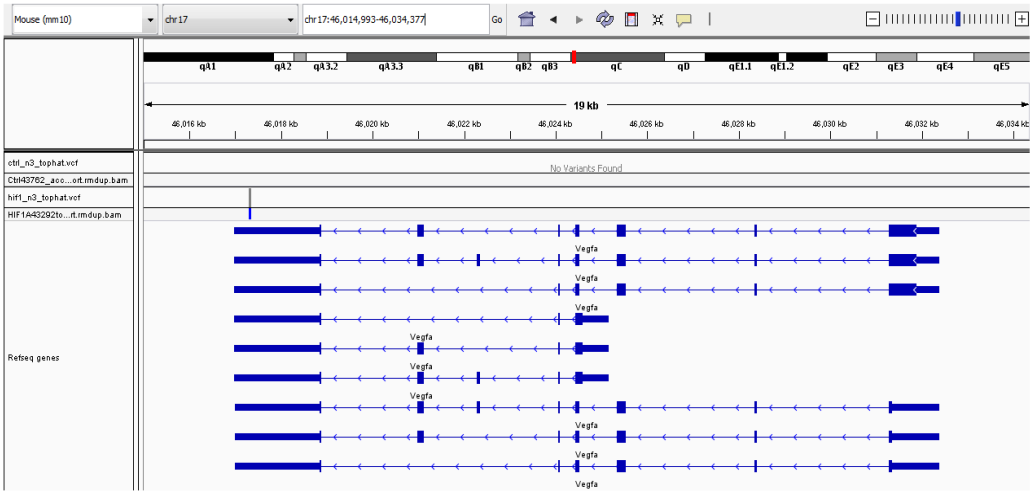
The effect of other SNVs identified in TRACK mice which were predicted to alter splicing were examined in the Cuffdiff isoforms output. These transcripts were *MIX1*, *SLC16A3*, *DYNII1*, *VPS8*, *ZBTB38*, *HILPDA*, *PGK1* and *CDC58*. The transcript *DYNII1* showed alternative splicing in one exon but that could be artefact as this is not supported by many reads. In contrast, the *PGK1* gene is significantly over-expressed (2.9FC, $q<0.05$) in TRACK. The *ZBTB38* transcript showed differential

promoter usage, whereas the *Hilpda* transcript was shorter in TRACK as compared to control mice (Fig. 2.11 & 2.12)

NDUFA4L2



VEGFA SNVs



CES1d SNVs

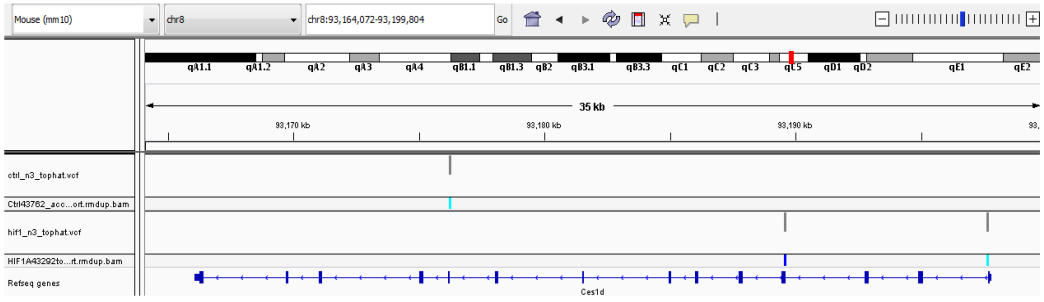
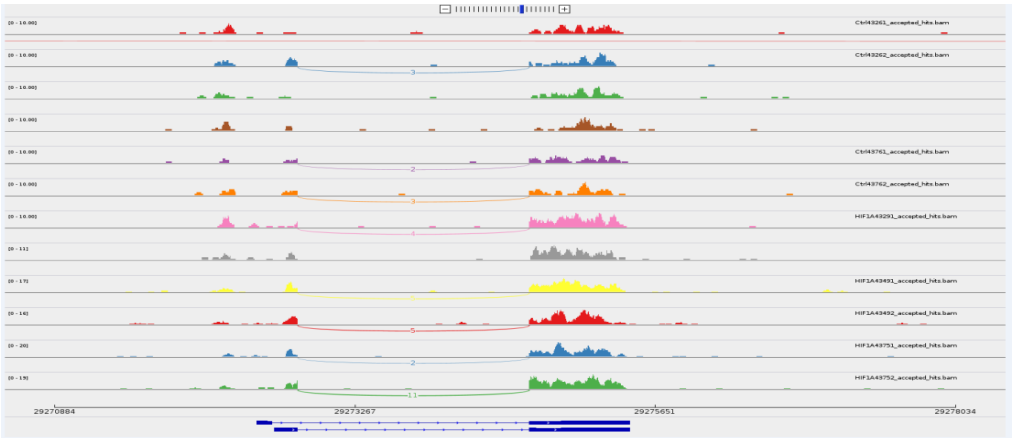
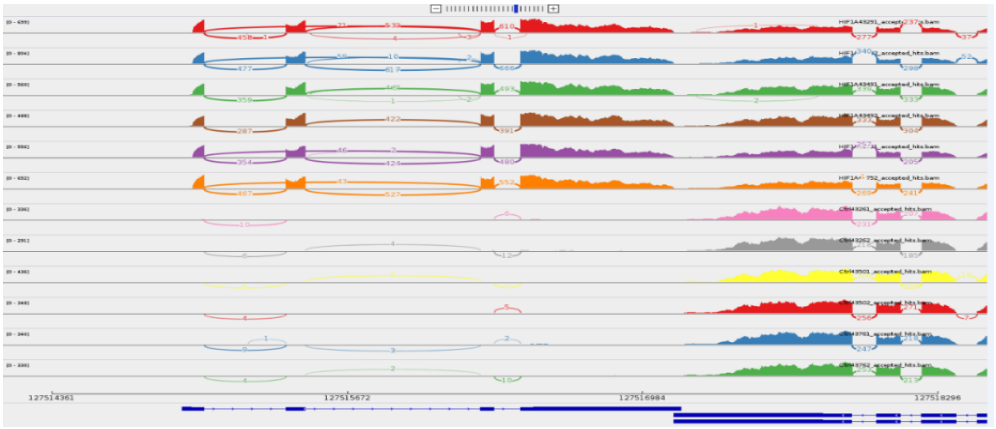


Figure 2.8: Splicing variants shown by SNVs affecting the known ccRCC transcripts and SNV identified in TRACK mice on Integrated Genome Viewer. Only *CES1d/ CES3* gene showing splicing variants whereas *NDUFA4L2* and *VEGFA* genes have no splicing variants

Hilpada



NDUFA4L2



ZBTB38

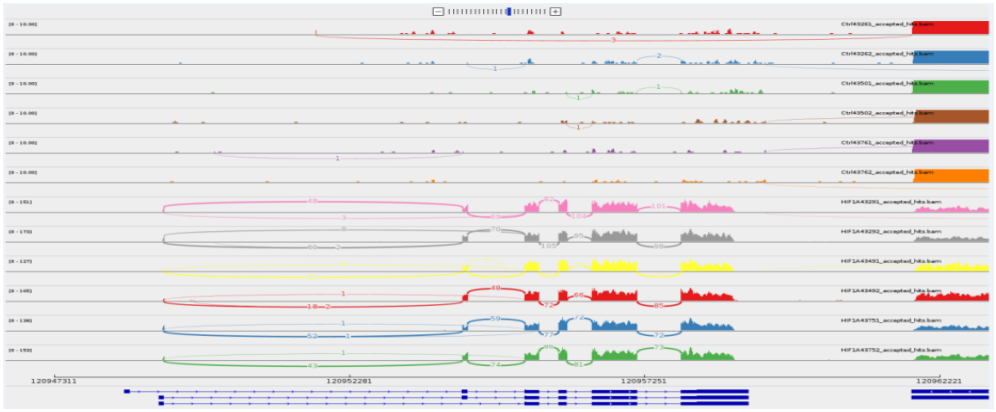
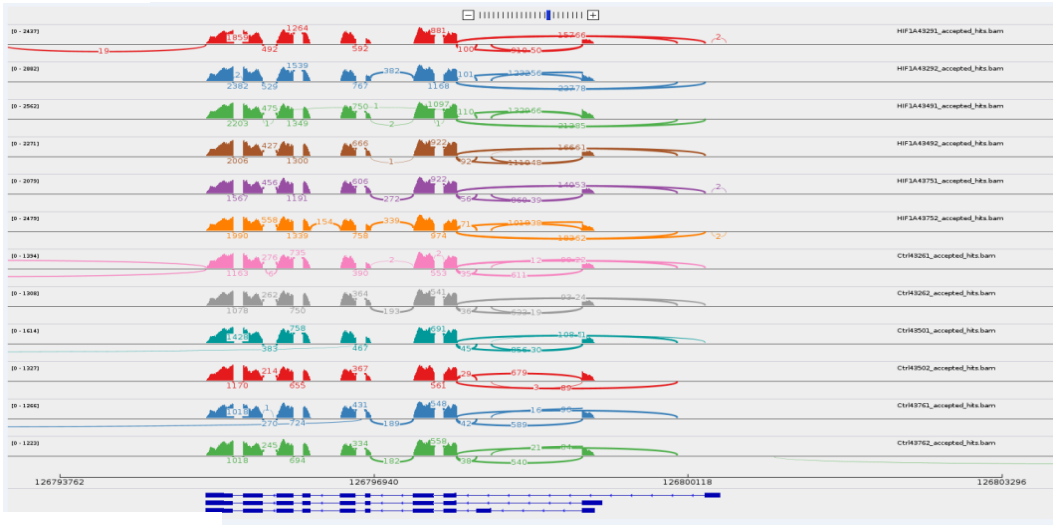
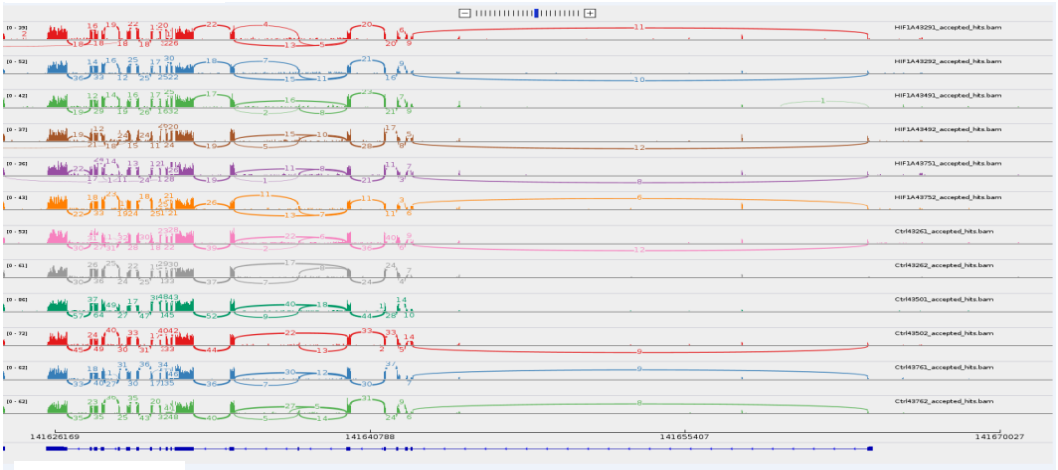


Figure 2.9: Comparison of SNVs predicted to alter splicing to the Cuffdiff isoforms output using IGV-generated Sashimi plots. *Hilpada*, *NDUFA4L2* and *Zbtb38* are showing significant expression and splicing variation as well.

CDC58



PLEKHM2



ALDOA

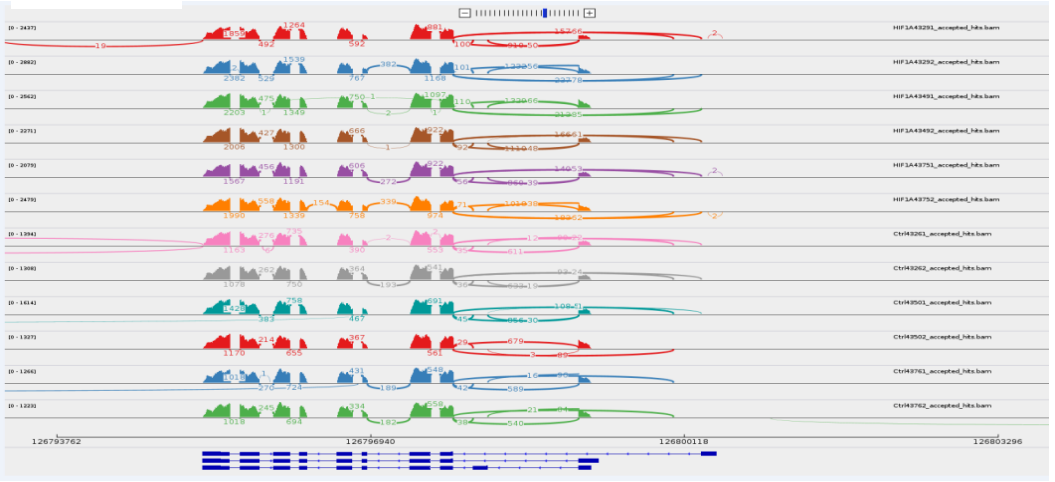


Figure 2.10: Comparison of SNVs predicted to alter splicing to the Cuffdiff isoforms output using IGV-generated Sashimi plots. *CDC58*, *PLEKHM2* and *ALDOA* transcripts are showing significant expression and splicing variation.

To check whether *HIF-1α* over expression induce fusion transcript, MapSplice was used to identify splice variants and/or fusions transcripts in control and TRACK and novel insertion/deletions. Only two Non canonical genes were different in TRACK (114 genes) in comparison to control (98 genes). The analysis did not identify novel splicing, fusion or insertion/deletion events in the TRACK mouse kidney.

2.4 Discussion

Research involving use of human ccRCC cell lines, ccRCC specimens of humans, murine models of ccRCC, and mouse transplants revealed the involvement of *HIF-1α* and *HIF-2α* in the progression of ccRCC (Gudas et al., 2014). Also literatures confirmed that the transcription factor HIF-1α (Hypoxia inducible factor alpha) constitutively expressed in many ccRCC and is responsible for the regulation of hypoxia–responsive genes (Harris, 2002, Semenza, 2003). In a recent study, ccRCC was induced by *HIF-1α* in transgenic mice. Therefore to study ccRCC cancer, recent developed murine model called TRACK (the transgenic cancer of the kidney) used which express *HIF-1α* constitutively precisely in renal proximal tubules recapitulates several pathological and molecular features of early human ccRCC (Fu et al., 2011). For example, expression of *CA9, Glut1*, and *CD31* (marker of angiogenesis) is highly raised in TRACK kidneys, and similarly human ccRCC exhibit high expression of these protein (Hui-Stickle et al., 2005, Jha et al., 1992). It is reported that TRACK mice resemble closely to the patients of VHL disease, comprising of deformed structure of tubule, multiple renal cysts and increased clarity of cells cytoplasm with increased deposition of lipid, glycogen, and early onset of ccRCC.

Based on this knowledge, we hypothesized that over-expression of *HIF-1α* increased genome instability, manifested by increasing mutation rate and aberrant transcriptional splicing and fusion events, in ccRCC. In the present study we used next generation RNA sequencing to identify single nucleotide variants (SNVs) in the TRACK ccRCC model relative to wild-type control mice.

The Illumina Hiseq technology usually produces fragments of short sequence (50–200 million) and 32–100 bp reads per run. The novel splice variants of genes can be identified by using the level of gene expression in these fragments of reads. Most

important process in RNA sequencing is mapping of NGS 'reads' to the reference sequence. Two important aims of RNA-Seq experiments can be achieved by mapping to the genome; First identification of unique transcripts (from the field of covered areas in the mapping) and second, assessment of abundance of transcripts in the mapping (from their depth of coverage). Many alignment programs have been developed in the recent past including Eland (Cox, 2007, unpublished material), MAQ (Sutherland and Alexander, 2012), RMAP (Hoste and Kellum, 2006), , SeqMap (Pisoni et al., 2008), SOAP (Sutherland and Alexander, 2012), ZOOM (Chertow et al., 1995) BFAST (http://genome.ucla.edu/bfast), CloudBurst (Schatz, 2009) Mosaik (http://bioinformatics.bc.edu/marthlab/Mosaik), MOM (Eaves and Gao, 2009), NovoAlign (http://www.novocraft.com), PASS (Goldstein, 2011), ProbeMatch (Jung Kim et al., 2009), ReSEQ (http://code. google.com/p/re-seq) and SHRiMP (http://compbio. cs.toronto.edu/shrimp). However all the above mentioned programs require a massive memory to form an index huge human genome and also to increase the sensitivity any sequencing error rates. In addition, the certain mapping strategies (Duzova et al., 2010, Vachvanichsanong et al., 2006, Palmieri et al., 2009, Schneider et al., 2010) comprise alignment measures to localize Illumina sequence to identified exons in the genome. Therefore in the present study the alignment was done with BWA (Burrows–Wheeler-Transform) and TopHat, as these are competent algorithms for read-mapping. Both TopHat and BWA intended to align reads from an RNA-Seq experiment to a reference genome whereas BWA do alignment without relying on known splice sites. Both TopHat and BWA can identify approximately 2.2 million reads/CPU hour. Therefore it was possible to complete the entire RNA-Seq experiment on a standard desktop computer in less than one day. The analysis for the SNVs were more robust as including three mice per group and variants identified by

both BWA and TopHat alignment that alter coding sequence are included. Three genes *GC, ZDHH4* and *CES3* were unique to TRACK out of which only *CES3* gene showed mutations in human cancer.

Altered cell metabolism is a characteristic feature of ccRCC disease (Linehan et al., 2010, Hanahan and Weinberg, 2011a). Loss of *VHL* expression is responsible for enhanced expression of *HIF-1α* and *HIF-2α* in ccRCC. This result into increased expression of HIF-responsive genes involved in glucose uptake, enhanced glycolytic metabolism and decreased mitochondrial respiration (Semenza, 2003). The present study reveals the potential role of a novel *CES3* mutation in renal clear cell carcinoma (ccRCC).

CES3 (carboxylesterase 3) gene is an orthologue of human *Ces1d* also known triglyceride hydrolase. It is involved in lipolysis by hydrolyzing stored triglycerides into fatty acids and enables both uptake as well as incorporation of nutrients into biomass. Proliferating tumour cells needs large amounts of fatty acids and cholesterol. Several literatures confirmed that it is achieved by hyperactivation of *de novo* lipogenesis in various types of neoplasia (Vitols et al., 1990, Pannu and Nadim, 2008, Palmieri et al., 2010, Andreoli, 2009, Huang et al., 2016). Cancer cells can also use exogenous FAs by converting it to lipids that are vital for proliferation and pro-tumourigenic lipid signalling (Arana et al., 2010, Louie et al., 2013). Present finding can easily relate to the "clear" histological subtype of renal cell carcinoma (Zappitelli and 2009), which significantly correlated to *HIF-1α* expression as *HIF-1α* showed enhanced expression of lipin 1, a phosphatidate phosphatase involved in the final step of triglyceride biosynthesis (Lafrance et al., 2010).

Huang et al, presented link between obesity and cancer and proposes a significant relationship between the host lipid metabolism and tumourigenesis (Huang et al., 2016). They illustrated increase VLDL production and impaired VLDL/LDL turnover by tumour leads to hyperlipidemia in mice and genetic ablation of *Ces3/ TGH* in mice caused reduced tumour growth by a low source of cholesterol-rich lipoproteins to the tumour (Huang et al., 2016). However, the exact cellular mechanisms which link obesity with cancer incidence, recurrence, progression and death remain to explore.

Genetic instability is driven by genetic changes like deletion or insertion and base substitution of few nucleotides at nucleotide level whereas translocation, gain or loss of chromosome and its rearrangements (Venkatachalam et al., 2010). In ccRCC altered *VHL* gene leads to over expression of *HIF-1α* which subsequently increase *HIF* targeted genes such as *NDUFA4L2* which is one of the most expressed gene in almost all ccRCC samples compared to healthy kidney tissue. In the present study only *CES1d/CES3* is altered by mutation whereas other interesting transcripts *HIF-1α, HIF-2α, VHL, NDUFA4L2,* and *VEGFA* showed no alterations. In present study we did not validate the SNVs. Also these are kidney specific transgenes, therefore these SNVs effect are local and will not affect the germline. So it should consider these mutations like the mutations that arise in patients' tumors, rather than being germline mutation.

Several studies provide the information regarding association of hypoxia and gene amplification (Du et al., 2011, Parikh et al., 2011, Wheeler et al., 2008, Zappitelli et al., 2007). Gene Amplification is one of the most recurrent modifications noticed in cancer cell and is linked with over expression of several oncogenes hence associated with tumour progression (Bindra and Glazer, 2005). In present study analysis using TCGA genome dataset on cBIO portal showing *CES3/CES1d* is amplified in 5% of

human ccRCC and there is high expression level of *CES3/ CES1d* in tumour cell in comparison to normal cell when analysed on cancer genome browser.

The final objective in this chapter was does *HIF-1α* over-expression induce fusion transcript? Fusion transcripts can arise from the joining between two otherwise separated genes due to insertion, deletion, translocation or inversion. These genes are frequently found to function as oncogenes playing major role in progression of many cancers. Fusion transcripts can arise as a result of chromosomal insertion, deletion; inversion or translocation that joins two otherwise separated genes. Fusion genes are often identified as oncogenes. These genes usually play important role in the development of many cancers. Trans-splicing is an event that involves ligation of two different primary RNA transcripts. Both fusion genes (DNA level) and trans-splicing events (RNA level) can form fusion transcripts. Several online tools viz Bellerophontes, Chimerascan, FusionHunter, JAFFA, MapSplice, and TopHat-Fusion are available. In present study MapSplice tools were used to analyze. Literature suggested that MapSplice can detect maximum number of true fusions as well as a large number of false fusions. There are many others tools like JAFFA and Chimerascan that are advanced tools specifically with minimum false fusions. But these tools are working well for paired-end sequences with longer read length whereas MapSplice can be applied to both short (<75) and long reads (≤75 bp) of single-end sequences with less false discovery (Kumar et al., 2016). There were no significant results were observed between Control and TRACK mice for canonical, Non-canonical and semi canonical splicing performed by MapSplice. Therefore it is possible to conclude that *HIF-1α* over-expression appears not to be pro-mutagenic in TRACK mice.

Further, Cuffdiff was used to analyze differential gene expressions with 2 fold change and found few transcripts are significantly expressed and have splicing variants as well. These are *NDUFA4L2, Hilpda, Zbtb38, Cdc58, Plekhm2, Aldoa, Mix1, Slc16a3, DynII1* and *VPS8* out of which genes some of them showed splice junctions or variation on Sashimi plot software were *NDUFA4L2, Hilpda, Zbtb38, Cdc58, Plekhm2* and *Aldoa*. Although the SNV pipeline has identified a putative variants in splice acceptor site of *NDUFA4L2,* the Sashimi plot suggest it does not affect splicing for *NDUFA4L2*. The hypoxia-inducible lipid droplet-associated (*Hilpda/Hig2*) is a unique Peroxisome proliferator-activated receptors target gene which have role in hepatic lipid/ triglyceride metabolism (Linehan et al., 1995). In the present study Integrated Genome Viewer showed shorter *Hilpda* transcript variants in control as compared to the TRACK. *Zbtb38* gene encoded a zinc finger transcriptional activator that binds methylated DNA and associated with apoptosis in some cell types. This present study revealed its differential promoter usages as increase in *HIF-1α* appears to alter the transcriptional start site of *Zbtb38* in TRACK. *Zbtb38* have 10 possible alternative promoters along with 5 non-overlapping substitutive last exons and 6 validated alternative polyadenylation sites. The link between these genes and carcinogenesis of ccRCC needs to be further investigated.

Chapter 3: Investigation of potential mechanistic links between AKI and kidney Cancer

3.1 Introduction

Acute kidney injury (AKI) is most frequently encountered condition which has a very high risk death of the patient. It has been observed that in relation to time there has been a steady increase in the cases of AKI (Xue et al., 2006, Hsu et al., 2007). Concurrently, the prevalence of chronic kidney disease (CKD) has also been increasing (Coresh et al., 2007). Over the past several years, increasing evidence from experimental animal models and human studies indicate that AKI can result in irreversible damage of kidney, including CKD and it is also reported to damage organs other than kidney (Basile et al., 2001, Basile, 2004).

Historically, various measures have been used to determine the functional status of the kidney and define these abnormal functions which can be used as a guide in the diagnosis of the disease. Currently there are as many as 30 definitions cited in the literature for defining acute failure of kidney (Coresh et al., Kellum et al., 2002). The definitions exist to define disease of various orders of severity. It defines severe form of disease like ARF where dialysis is must for survival to less severe form of disease where there is slight increases in serum creatinine (Mehta and Chertow, 2003). This wide array of definitions have helped place the patients in a wide spectrum ranging from mild to highly severity allowing a subset of severely ill patients (requiring dialysis) to be distinguished from other patients. (Warnock, 2005). The chances of survival of the patients are directly correlated to the severity of kidney injury. The Acute Kidney Injury Network (AKIN) utilizes AKI as an abnormality of the structure or function or markers that describe the injury status of the patient such as alterations

in the tests of urine, blood, or tissue or on the basis of the image studies conducted within a span of three months. In normal condition urea, creatine and wastes that arise from other metabolic processes are eliminated by the kidney, however in AKI the kidney is unable to perform such eliminations and they are retained in the body. In patients suffering from severe AKI there may exhibit normal urine production or there may be alterations in the urine volumes such as production of excessive urine or little amount of urine (oliguria) or no urine production (anuria) (Mehta et al., 2007).

3.1.1.1 AKI causes and progression

Acute kidney injury may arise because of various reasons such as reduction in the perfusion efficiency of renal or intra-renal regions, damage to renal tubule because of toxic substances or blockage, swelling, tubule interstitial inflammation or significant decrease in the filtration capability of renal glomerulus (Thadhani et al., 1996)

Toxins or ischemia (reduction in the blood supply to tissue or organ) usually lead to sepsis in the kidney and result in AKI. When the kidney is affected by both toxins and ischemia it results in a more severe acute kidney injury which involves sepsis, AIDS or hematologic cancers. Pre-existing kidney disease predisposes patients for AKI and related to this AKI risk is proportional to CKD stage (Parfrey et al., 1989, Nash et al., 2002, Hsu et al., 2008, Ishani et al., 2009, Khosla et al., 2009, Okusa et al., 2009). On the other hand, patients who had chronic kidney disease (CKD), the kidneys are more prone to damage which result in the progression to End Stage Renal Disease (ESRD) (Hsu et al., 2009, Khosla et al., 2009, Okusa et al 2009). Through clinical studies it has been reported that progression of AKI may also result in patients with no previous kidney disease. Here the patient may not recover completely resulting in residual damage which may slowly progress to AKI (Finn, 1993, Gueler et al., 2004, Hsu et al., 2007, Ponte et al., 2008, Snoeijs et al., 2010, Coca et al., 2009)

In kidney, ischemia reperfusion injury (IRI) contributes to pathological conditions including AKI that is a clinical syndrome with rapid kidney dysfunction and high mortality rates (Hoste et al., 2006, Kellum et al., 2011). The mechanisms involved in the pathophysiology of IRI in kidney is not simple and involves several complicated pathological pathways such as neutrophils activation, ROS and intermediates of inflammatory pathways (adhesion molecules and a variety of cytokines). Studies have demonstrated the beneficial effects of different agents in combat with IRI (Dosluoglu et al., 1993, Erkasap et al., 2004, Korkmaz and Kolankaya, 2009, Kucuk et al., 2009, Ihtiyar et al., 2011). It has been stated that IRI is the dominantly responsible for causing not only in native but also in the transplanted kidneys (Jang et al., 2009). Renal IRI triggers an inflammatory cascade that involved in more renal damages, so inhibition of inflammatory responses is a therapeutic approach to protect renal tissue (Jang et al., 2009, Stroo et al., 2010).

The advancements in the acute kidney injury in patients are because of the permanent damage of the kidney on account loss nephrons and left over scars in these tissue regions. Various altered physiological conditions involved in hypertension, excessive load of calories, diabetes mellitus, and aging can itself initiates the injury of kidney or it may have a cumulative effect of the pre-existing CKD. In such condition the hemodynamics of the kidney is disturbed, increases the physiological and metabolic stress/load and this aggravates the pre-existing damage of kidney (Bidani and Griffin, 2002, Caramori and Mauer, 2003, Abrass, 2004a, Johnson et al., 2005, Nguyen and Hsu, 2007, Fioretto et al., 2008, Griffin et al., 2008, Bakris and Ritz, 2009, Balakumar et al., 2009). Multiple structural (Nath, 1992, Bidani and Griffin, 2002, Gueler et al., 2004, Johnson et al., 2005, Eddy and Neilson, 2006, Nangaku, 2006, Schlondorff, 2008), functional (Caramori and Mauer, 2003, Abrass, 2004a, Abrass, 2004b, Johnson

et al., 2005, Nguyen and Hsu, 2007, Fioretto et al., 2008, Griffin et al., 2008, Balakumar et al., 2009), and signalling abnormalities (Megyesi et al., 1999, Nakagawa et al., 1999, Park et al., 2001, Okusa, 2002, Andreucci et al., 2003, Spurgeon et al., 2005, Jo et al., 2008, Geng et al., 2009, Li and Durbin, 2009, Zeng et al., 2009) can affect diverse kidney cell types during progression (Vaidya et al., 2008).

The kidney has the capability to recover back from various degrees of functional renal damages. For this to happen there has to be a sequence of events that must be followed after the injury. These include the spreading and migration of the epithelial cells to conceal the portion of the basement membrane that was exposed, regeneration of tissue by dedifferentiation and multiplication of cells. Once the cell number is restored the cells undergo differentiation to allow achieve ultimate functionality of nephron (Bonventre, 2003). Various studies support the thinking that there exists a dynamic association between repair of tissue, disease advancement or relapse of renal damage (Witzgall et al., 1994, Vaidya et al., 2003a, Vaidya et al., 2003b, Vaidya et al., 2008, Remuzzi et al., 2006). In circumstances where the repair of nephrogenic tissue is delayed or is inhibited, there occurs advancement in the injury of kidney and thus result in chronic kidney disease. However if the repair process is not delayed (timely treatment), the advancement of injury is halted, tissue gets chance to the undergo regression and recover (Vaidya et al., 2003a).

3.1.1.2 Pathophysiology and mechanisms of AKI

A variety of disturbances in the physiological mechanisms as a result of infection or disease can lead to AKI in response to toxins or ischemia.

These could be attributed to (*a*) hampering of self-regulation and increase in the constriction of renal blood vessels, resulting in decreased renal perfusion, (*b*) loss of tubular renal function and death of cells by necrosis or programmed cell death, (*c*) shedding of the outermost layer of cells comprising of both viable and dead cells which result if obstructions within tubules, (*d*) Abnormality in the transportation because of metabolic changes result in abnormal balance of glomerular tubule, and (*e*) inflammation of interstitial tissue and vascular jamming as a result of local production of mediators of inflammation (Bonventre, 2003, Schrier et al., 2004).

Studies in the recent past has been able to throw light on various important links like AKI, CKD and ESRD (Coca et al., 2009, Hsu et al., 2008, Hsu et al., 2009, Ishani et al., 2009, Khosla et al., 2009, Lo et al., 2009, Okusa et al., 2009, Waikar and Winkelmayer, 2009, Wald et al., 2009, Thakar et al., 2009). These studies support hypothesis that collaborative interactions between AKI and CKD lead to huge alterations in the kidney pathophysiology and aggravate the disease condition that ultimately progress to ESRD.

When the response of these injuries is observed on the cell, it could be observed that there is loss of integrity of cytoskeleton, loss of polarity of cell, mis-localization of various molecules responsible for cell adhesion, other membrane proteins (Na^+K^+ ATPase and β-integrins), that result in dislodging the brush border proximal tubule, cell death by necrosis and apoptosis (Zuk et al., 1998). When the injury is more pronounced, it results in dislodging or removal of not only non-viable cells but also viable cells which results in exposure of basement membrane (only barrier between peritubular interstitium and filtrate (Bonventre and Zuk, 2004).

3.1.1.3 Animal models of AKI

Although use of animals in experimentation is a matter of debate and is rightly matter to rigorous restrictions by law and ethics. However, it is important to perform pre-clinical trials on these animal models before the technology could be tried on humans. There has been development of therapeutic treatments because our understanding of disease pathophysiology has improved tremendously because of usage experimental models. This becomes altogether more important in renal IRI studies because there are only few available *in vitro* models (Lange et al., 2005, Togel et al., 2007, Choi et al., 2009, Pino and Humes, 2010, Hara et al., 2011, Giraud et al., 2011). A large number of studies on renal IRI is dedicated on rodent models which focused of earlier concepts involved various protocols for reperfusion (Bedi et al., 2010). Although the rodent model is cheaper and easier to manage in the laboratory than large animal models, studies have been limited because of challenges in the extrapolation of rodent data to human. Earlier the gene knock out studies were restricted go rodent models, however now such knock out or genetic manipulations can be performed in pigs which gives an opportunity to widen our perspectives (Giraud et al., 2011). Non-human primates, dogs and pigs are the three most widely used experimental models for transplantation pre-clinical trials. Use of these models has helped tremendously to evaluate the efficacy of pharmacological therapy and to evaluate devices incepted in medicinal preclinical trials. Certain attributes such as resemblance in size, physiology, development of organ and manner in which the progression of disease takes place in pig is similar to humans, therefore pig is considered as a superior model that can be used for research on human diseases (Lunney, 2007). Because of these similarities there is basic information available on the recovery of graft and inflammatory mechanisms/response that can be utilized for i*n vivo* studies. The kidney of humans

and pigs are structurally similar exhibiting multi-lobular structures, however dog and rodent kidneys exhibit uni-lobular kidney. In addition, the large body and organ size of the European large white pig allows performing various surgical in a manner performed on humans, it permits multiple time collection of the peripheral blood from pig, or conduct biopsies. Therefore, pig becomes an important model for medical training as well as for evaluation of preclinical technologies. Furthermore, pig models are relevant substitutes to non- human primate models which offering promising perspectives with developments of transgenic modulation and marginal donor models enabling data extrapolation to human conditions (Lieberthal and Nigam, 2000, Kirk, 2003, Lunney, 2007, Dehoux and Gianello, 2007, Simmons et al., 2008, Giraud et al., 2011, Gardner et al., 2014, Gardner et al., 2016).

Similarity in renal anatomy and vascular bed of porcine to human kidneys, give advantage to these models to be applicable to assess the renal response following injury like renal tubular inflammation and repair processes (Wen et al., 2010). Comparison on the basis of renal embryology, anatomy, architecture and lymphatic pattern, ability to concentrate urine, and sensitivity to ischemia of porcine suggest that the pig kidney may be more similar to the human kidney than those of the dog or rodent. Therefore it is considered as superior models to study ischemia/reperfusion consequences (Schmidt-Nielsen and O'Dell, 1961, Killion et al., 1993, Lieberthal and Nigam, 2000, Simmons et al., 2008). The genome was sequenced and characterized for porcine model under the auspices of the Swine Genome Sequencing Consortium (Schook et al., 2005, Archibald et al., 2010). Genomics tools for *Sus Scrofa* including microarrays have been available since 2005 (Zhao et al., 2005). To portray a preclinical model of chronic ischemic wound (Roy et al., 2009) and also to study the

effect of hypoxia on stem cells (Wang et al., 2010b) this advance technology has been used.

3.1.2 Hypoxia and AKI

It has now been well established that the primary reasons which can lead to AKI include (i) ischemia: insufficient blood supply to an organ or to a specific part of the body, (ii) hypoxia: insufficient supply of oxygen to the tissue or (iii) nephrotoxicity: exposure of body to the toxins or drugs that cause kidney damage (Basile et al., 2012). The various genes such as those involved in the oxygenation of tissue, metabolism of cell and cell survival are important protective genes gets affected by the hypoxic conditions. Hypoxia-inducible factors consist of subunits α & β and exist as a heterodimer which has significant role in the expression of genes under decreasing partial pressure of oxygen (Gunaratnam and Bonventre, 2009). There occurs inhibition of HIF-prolyl-hydroxylase under reduced oxygen conditions. This permits quick accumulation of *HIFα* which can readily combine with *HIFβ* to for a heterodimer (*HIFαβ*). This heterodimer is then transported to the nucleus where it brings about the activation of large number of genes that help to adapt to hypoxic conditions

When AKI is induced by ischemia, pathogenic bacteria or its toxins (sepsis), hypoxia plays an important role. It induces an adaptive response in renal kidney that could minimise the impact of damage. Therefore, the effect of damage that otherwise would have been lethal is reduced to sub-lethal response where cell continue to survive (Naito et al., 2009). One of such adaptation includes the distinguished capability of kidney to reduce oxygen intake for tubular transport, thus encourages renal

oxygenation. Through this adaptation the cellular integrity is not lost during hypoxia (Heyman et al., 2012).

It is believed that the hypoxic stress in kidney may have developed due to (i) suppression of regional perfusion, (ii) enhanced use of oxygen in tubular transport or (iii) because of both i & ii. Reduction in the physiological partial pressure of oxygen in the medullary region of kidney makes it susceptible to hypoxic damage. These damages are observed in the S3 region of proximal tubule in experimental warm ischemia and reperfusion or in the thick uphill limbs of medullary region (mTALs) of AKI models (Heyman et al., 2000, Rosenberger et al., 2006, Heyman et al., 2010).

AKI arising as a result of various reasons stated above can lead to chronic renal disease, renal hypoxia and tubular hypoxic stress. The various adaptive responses to the hypoxic condition may involve redistribution of blood flow within renal chambers, reduction in glomerular filtration rate, and reduced tubular transport. Microvascular injury and dysfunction are known to influence pathogenesis of acute kidney injury. It created a feed-forward loop of hypoxia and lead to formation of reactive oxygen species (Zager et al., 2007). In order to prevent AKI and weaken the effect of hypoxia on renal parenchyma, it is important to restore the lost microvascular function. One of such measures that have been observed during sepsis in mice is suppression mitochondrial oxygen utilization. To achieve this, genes involved in the biogenesis of mitochondria or mitochondrial respirations are down-regulated and are most likely facilitated by suppression of transcriptional co-activator (*PGC-1a*). The net balance between the oxygenation and consumption of oxygen in the altered microcirculation during AKI reflect the extent of damage. The understanding of the processes that enhance oxygen or reduce its consumption in the

organ or site of altered microenvironment would help to prevent AKI (Heyman et al., 2012).

3.1.3 Hypoxia and ccRCC: How hypoxia in AKI may predispose to ccRCCS

Most patients who present with advanced RCC have a poor prognosis because RCC easily metastasizes and improved therapies have been limited (Linehan et al., 2004; Zini et al., 2009; Jemal et al., 2010). A common risk factor are shared by both Acute kidney injury and chronic kidney, also it have common disease modifier. Chronic kidney disease can develop without pre-existing acute kidney injury, on the other hand occurrence of chronic disease leads to development of AKI. Hence presence of either or combination of AKI or CKD may result in ccRCC (Goldberg and Dennen, 2008; Coca et al., 2009).

AKI often complicates CKD especially in stages 3 to5 as permanent decrements in kidneys have been noticed after single or repeated episodes of AKI/ARF in Persons with or without pre-existent kidney disease. AKI represents a substantial risk factor for progressive CKD and then ccRCC. A steeper decline in renal function can be noted due to a single episode of of AKI than normally expected from aging alone. Hence patient must be closely monitored, who develop severe AKI or recover slowly from AKI even when the *eGFR* returns to baseline.

3.1.4 Biomarkers for AKI and ccRCC

The diagnosis and treatment of AKI and ccRCC patients depend on the potential of biomarkers used. Yet to date no very specific and useful tumour markers for diagnosis and follow-up of AKI has been identified. Studies reveal the two factors should be taken into account while establishing an effective therapies for AKI: (1) development of specific, sensitive and consistent biomarkers for early

prognosis/diagnosis of AKI in preclinical and clinical studies and (2) expansion and authentication of high-throughput pioneering technologies that consent rapid multiplexed detection of multiple markers at the bedside (Vaidya et al., 2008).

Urine, components of serum, imaging studies or other measureable parameter can be Accessible markers of AKI. To detect AKI in early stage, the molecular/ genes are the utmost promising markers. These markers are beneficial and convenient tools for the earlier diagnosis, identification of mechanism of injury, and assessment of site and severity of injury. Optimistically, these markers will be ascertain to be advantageous in enabling early diagnosis, supervising aimed intervention and finally checking disease progression and resolution when used in one or more, either alone or in combinations

3.1.5 Hypothesis

Collectively the published evidence leads to the hypothesis that AKI related hypoxia can induce several molecular and pathological changes which may resemble processes in ccRCC and may predispose to ccRCC risk. To test this hypothesis, RNA sequencing was used to compare the transcriptome of animal models of ccRCC and AKI and human ccRCC tumour specimens. This analysis has identified a key network of genes commonly deregulated in AKI and ccRCC.

3.1.6 cDNA library preparation and sequencing and quality processing:

Total RNA from control (n=3) and HIF-1α-over-expressing TRACK (n=3) transgenic mice were submitted to the genomics facilities at Weill Cornell Medicine (Fu et al., 2011, 2015) for single-end Illumina HiSeq RNA sequencing. Please see chapter (section 2.2.1) for detailed description of the processing of mouse RNA sequencing data. Total RNA from control (n=2) and AKI pig kidney samples (n=2) were submitted to the Barts and London Genomics center for Illumina RNA sequencing. For pig sample paired-end sequencing strategy was employed. In both instances a minimum of 20 million reads were obtained for each sample. Raw fastq sequences obtained from Ilumina NextSeq were analyzed using a local Linux cluster. The TrimGalore wrapper for Cutadapt and FastQC was used to trim adapter sequences and low quality bases (phred score <30). Quality-processed fastq files were aligned to the mouse and pig Illumina iGenome ENSEMBL reference sequences as appropriate.

3.1.7 Differential gene expression analysis

For differential gene expression analysis, the quality-processed fastq were aligned using TopHat2 to Illumina iGenome ENSEMBL annotated pig (Sscrofa10.2) and mouse (GRCm38/mm10 reference sequences and use for annotations (Trapnell et al., 2009). The resultant BAM alignment files (for each condition) with transcript annotation file were provided as input to Cufflinks/Cuffdiff (Trapnell et al., 2010) to assemble individual transcripts from RNA-Seq reads that had been aligned to the genome and predicted the relative abundances of each transcript by counting the number of reads that mapped to the genomic location of that transcript. Abundances were stated in FPKM which denoted the expected fragments per kilo base of

transcript per million fragments sequenced (Trapnell et al., 2010) for pig samples and RPKM for the single-end RNA sequencing analysis of the TRACK mice.

Transcripts were assembled by cufflinks were used by Cuffdiff to compared gene expression between two samples (Normal and TRACK), to identify differential isoform and differential promoter usage. The reads for each biological replicates were mapped independently. Only the comparisons with "q-value" less than 0.05 and expression fold change greater than two fold in the Cuffdiff output were taken as significant differential expression. The differential gene list was analysed for gene annotation (GO Analysis) and analysed in the Cancer Genome Atlas (TCGA) research network croc (KIRC) dataset (Creighton CJ, 2013).

The comparisons of differential gene expression identified after cuffdiff output analysis in AKI (IRx2) and TRACK ccRCC (TRACK) were done by Venn diagram analysis.

Venn Analysis: Cuffdiff output list of Control and Ischemic reperfusion pig (IRx2) were pasted (one element per row) in two lists box of Venny online software program (http://bioinfogp.cnb.csic.es/tools/venny/) and clicked the numbers to see the result and saved it by right click. Similarly, comparision of differential gene expression identified in AKI (Cntrl_IRx2) and TRACK ccRCC (TRACK) were done. The Common differential genes were identified for further analysis.

3.1.8 qRT-PCR methods

Total RNA from control and AKI pig kidney was isolated and converted into cDNA (provided by Professor David Gardner). In the present study, Cuffdiff a part of the Cufflinks package was used to compare differentially expressed genes of interest both

in AKI and cancer model conditions by rigorous statistical analysis.it shows result in mean FPKM /RPKM value. Genes were selected for validation on the basis of FPKM/RPKM and greater than 2 fold change. Post-Hoc test with Benjamin-Hochberg (BH) procedure was used and only $p>0.05$ was considered as significant expression. TaqMan PCR assays for used each gene target were performed in triplicate on cDNA as described (Nilsson et al., 2015). The following assays were employed for qRT-PCR analysis of pig samples: *Rbp4:* Ss03393811_g1; *MYC*:Ss03649118_sH; *EGR1:* Ss03373483_s1; *ASNS:* Ss04245795_m1; *ANXA2*: Ss03391794_m1; *TTR:* Ss03383377_u1; *FMOD:* Ss03373329_s1; *HRG:* Ss04328164_m1; *NQO1*: Ss04246170_m1; *SLPI:* Ss03392443_g1; *MMP7*: Ss03383345_u1; *SLC30A2*: Ss04321323_g1 and normalized to expression of *actin*: Ss03376160_u1. qRTCPR analysis of mouse samples employed the SYBR green method and the following primer pairs: *mMyc:* F: 5′-TCGACTCGCTGCTGTCCTCCGAG-3′, 5′-CCTCTTCTCCACAGACACCACATC-3′, and *mRBP4:*F:5′-CGCTCTGCAGTACTCCTGCCGCCTG-3′; R:5′-GCTACAAACTGTTTCTGGAGGGCCTG-3′; *mANXA2:* F:5′-GAAGGGCAAGGGGACTCGAGACAAG-3′; R:5′-GTGATCCACTGTGCTGAGCCCTTCAG-3′; *TTR:* F:5′-CAGTTCCTCGGTGCTGCCGGAACC -3′, R: 5′-CCGAGTCGTTTGGCTGGGATAACTC -3′ and *mβ-actin:*F:5′-GATCCACATCTGCTGGAAGG-3′, R:5′-AAGTGTGACGTTGACATCCG-3′. The identities of the amplified PCR products were confirmed by direct Sanger sequencing.

3.2 Results

The TopHat2 aligned sequences of samples of Ischemic perfusion pig (2) and control_saline sample (2) were processed for Cuffdiff (p>2 FC) and the results DEG were compared with DEG from control and TRACK. Venn diagram (Figure 3.1) showed that ischemic reperfusion pig and TRACK expressed 208 and 897 unique genes that account for 18.2% and 78.5% of total expressed genes. A cohort of 37 genes were found that was differentially expressed in both AKI and ccRCC disease processes that was 3.2 % total expressed genes gene list was shown (Figure 3.1). Twenty four genes out of 37 are upregulated in both AKI and ccRCC diseases that account for 88.8% of total common expressed genes whereas rest 13 genes plays important role in AKI. Specifically *APOA4* (616 FC), *ANXA2* (42 FC), *AZGP1(126 FC), CDKN1A (27 FC), MYC (10 FC), PFKFB3 (15 FC), RBP4 (89 FC)* and *TNFRSF12A* (29 FC) gene were highly expressed in ccRCC disease conditions than the AKI (Table 3.1).

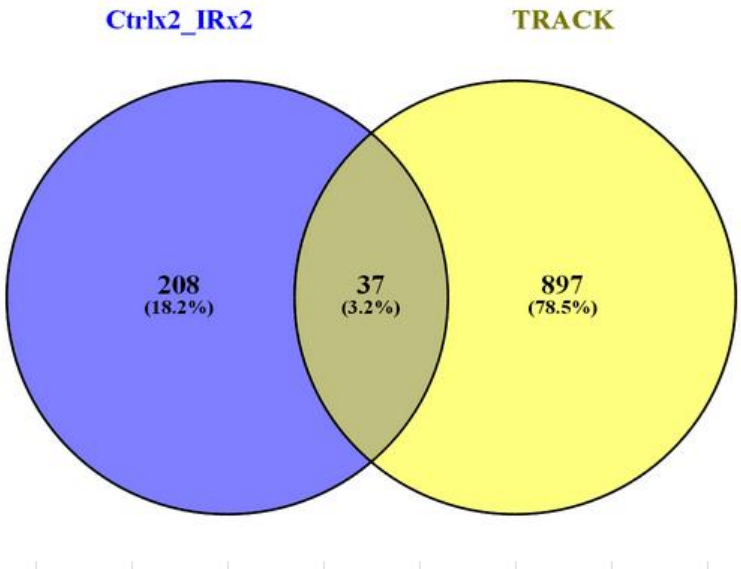


Figure 3.1: Venn diagram illustrating the analysis comparing differential gene expression identified in AKI (IRx2) and TRACK ccRCC (TRACK). The Ischemic reperfusion pig and TRACK expressed 208 and 897 unique genes respectively whereas 37 common genes were expressed

Table 3.1: Differential expressed genes in AKI and TRACK and those that are differential in the same direction in both conditions. Those expressed either higher or lower (24 of 37 genes) in both AKI and ccRCC are indicated in bold. Those tested by qRT-PCR are underlined.

Gene Name	Description	AKI FC	TRACK FC
ADAMTS15	ADAM metallopeptidase with thrombospondin type 1 motif 15	**1.49951**	**3.20449**
ANXA2	annexin A2	**1.28542**	**41.9997**
APOA4	apolipoprotein A-IV	**5.29525**	**615.95865**
ASNS	asparagine synthetase (glutamine-hydrolyzing)	**1.54427**	**13.01304**
AZGP1	alpha-2-glycoprotein 1, zinc-binding	**1.82234**	**125.6239**
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	**3.44959**	**27.2135**
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	**1.50236**	**4.13512**
CFTR	cystic fibrosis transmembrane conductance regulator	**2.28997**	**2.47838**
CHAC1	ChaC glutathione-specific gamma-glutamylcyclotransferase 1	**3.18296**	**2.457135**
EGR1	early growth response 1	**1.30804**	**3.84005**
ELF3	E74-like factor 3 (ets domain transcription factor, epithelial-specific)	**1.14585**	**8.93412**
JUNB	jun B proto-oncogene	**1.25884**	**6.94959**
MAFF	v-maf avian musculoaponeurotic fibrosarcoma oncogene homolog F	**1.84538**	**2.62204**
MYC	v-myc avian myelocytomatosis viral oncogene homolog	**1.69595**	**10.39858**
PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	**1.94903**	**14.77102**
RBP4	retinol binding protein 4	**3.41779**	**89.146399**
SFRP5	secreted frizzled-related protein 5	**1.80551**	**1.188181**
SLC34A2	solute carrier family 34 (type II sodium/phosphate cotransporter), member 2	**2.81532**	**4.479052**
STEAP4	STEAP4 metalloreductase	**3.77009**	**1.394278**
TNFRSF12A	tumour necrosis factor receptor superfamily member 12A	**2.91335**	**29.0058**
TOP2A	topoisomerase (DNA) II alpha	**1.3745**	**1.01847**
GPR98	adhesion G protein-coupled receptor V1	**-2.00281**	**0.952378**
PGLYRP2	peptidoglycan recognition protein 2	**-2.03643**	**0.19262**
SH2D1A	SH2 domain containing 1A	**-2.57441**	**0.356638**
ADAMTS4	ADAM metallopeptidase with thrombospondin type 1 motif 4	3.33206	0.330598
LYVE1	lymphatic vessel endothelial hyaluronan receptor 1	3.2555	0.3253674
SOX9	SRY-box 9	2.23761	0.262986
F2	coagulation factor II (thrombin)	-1.94491	80.03877
FMOD	fibromodulin	-1.18496	2.32629
HPD	4-hydroxyphenylpyruvate dioxygenase	-1.37219	51.85386
HRG	histidine-rich glycoprotein	-1.28518	8.91432
INMT	indolethylamine N-methyltransferase	-1.69061	425.073
POSTN	periostin, osteoblast specific factor	-2.67954	1.31486
SLC17A4	solute carrier family 17, member 4	-3.34523	2.46003
SLC4A11	solute carrier family 4, sodium borate transporter, member 11	-1.76829	1.063277
TTR	transthyretin	-3.68217	50.827

From cohort of 37 genes, several genes were selected for validation by TaqMan qPCR. The upregulated genes selected for validation were *MYC, ANXA2, EGR1, and RBP4* both in pig and TRACK model, whereas *FMOD, NQO1, MMP7, SLC30A2, HRG, TTR* and *SLPI* genes were validated in pig. Figure 3.2 showing the relative expression of *MYC, RBP4, ANXA2* and *EGR1* in WT (Wild type) and in TRACK. The genes *RBP4, MYC, ANXA2* were significantly expressed in TRACK than wild type whereas *EGR1* was not significantly differentially expressed.

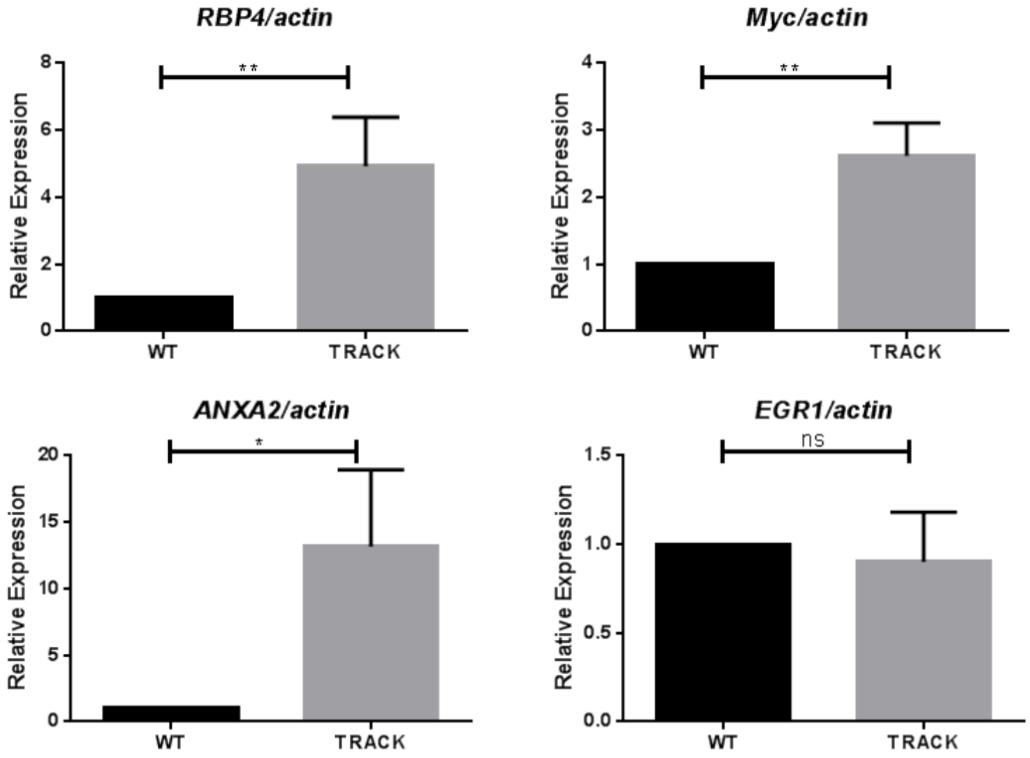


Figure 3.2: Quantitative qRT-PCR showing the relative expression (normalized to β-actin gene) of *RBP4, MYC, ANXA2* and *EGR1* in wild type (WT) and TRACK mouse. The data show significant expression of *RBP4, MYC* and *ANXA2* whereas *EGR1* gene was not significantly expressed in comparison to wild type. The relative expression levels were calculated by Pfaffl method and statistically analyzed by t-test with $p>0.05$ considered significant.

The relative expression of genes *MMP7, FMOD, RBP4* and *NQO1* were significantly expressed in pig AKI model in comparison to control pig where as other selected genes viz *TTR, SLPI, SLC30A2* and *HRG* showed insignificant relative expression compared to control (Fig. 3.3 &3.4). The relative expression was measured by Pfaffl method and the difference in expression level showing $p>0.05$ were taken as significant in one tailed t-test.

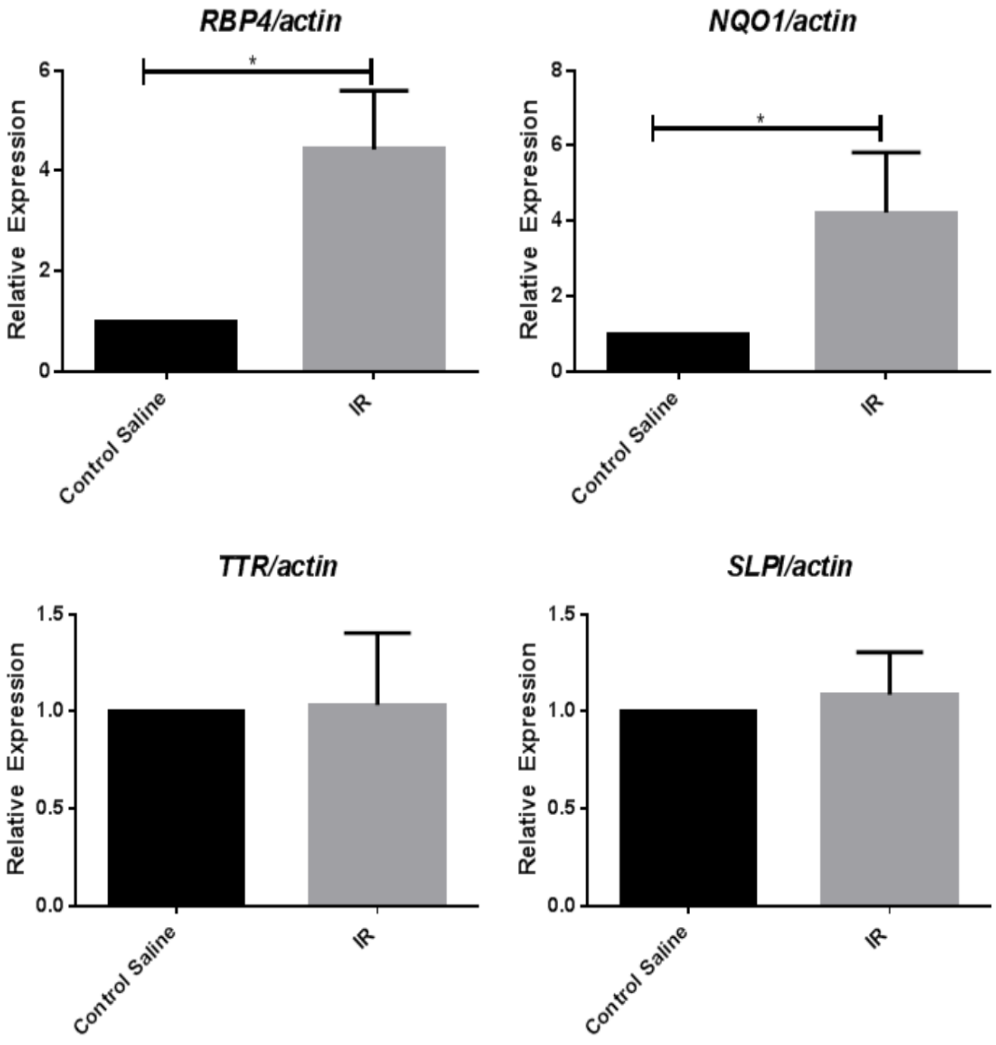


Figure 3.3: TaqMan quantitative PCR showing the relative expression (with respect to Actin gene) of *RBP4*, *NQO1*, *TTR* and *SLPI* in control saline and Ischemic reperfusion pig samples. The Data show significant expression of *RBP4* and *NQO1* whereas *TTR* and *SLPI* gene was not expressed in comparison to control. The relative expression levels were calculated by Pfaffl method and statistically analyzed by t-test with $p>0.05$ considered significant.

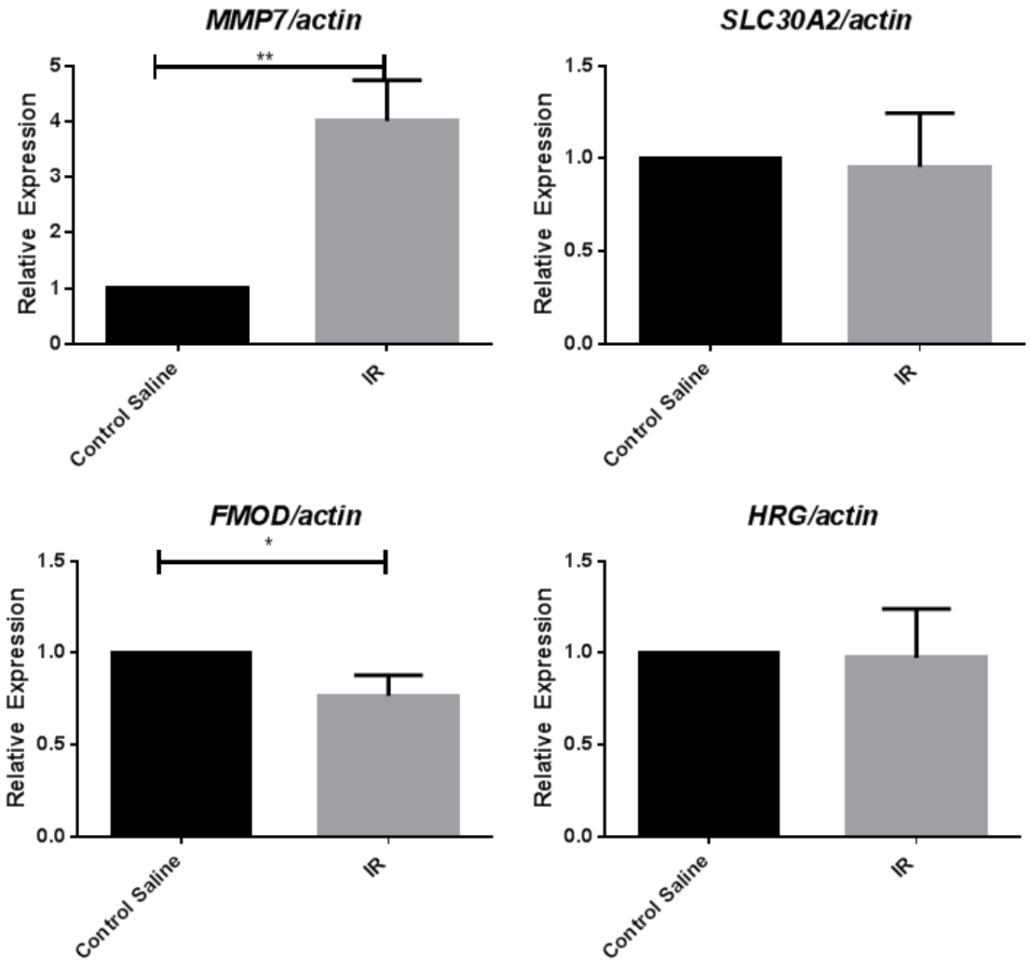


Figure 3.4: TaqMan quantitative PCR showing the relative expression of *MMP7*, *SLC30A2*, *FMOD* and *HRG* in control saline and Ischemic reperfusion pig samples. The Data show significant expression of *MMP7* and *FMOD* and whereas *SLC30A2* and *HRG* gene was not expressed in comparison to control. The relative expression levels were calculated by Pfaffl method and statistically analyzed by t-test with $p>0.05$ considered significant

Figures 3.5A to 3.14A show the comparison of cuffdiff analysis comparing gene expression between control & TRACK mouse, also similar analysis in control_Saline & Ischemic reperfusion. The analysis illustrate that all gene of interest *TTR*, *NQO1*, *SLC30A2*, *FMOD*, *RBP4*, *HRG*, *MYC*, *ANAX2* and *EGR1* are significantly expressed in pig except *SLPI*, where as in mouse (TRACK) apart from *MMP7* all genes were significantly expressed. Further the integrated data of pig and mouse were extrapolated with human data in TCGA KIRC gene accounting 606 patients. The

result showed that in human only two genes *MYC* and *ANXA2* were highly expressed in cancer patient than normal. Rest of the genes *TTR, NQO1, SLC30A2, MMP7, FMOD, RBP4, HRG, EGR1* and *SLPI* were expressed in Normal sample (Figures 3.5B to 3.14B).

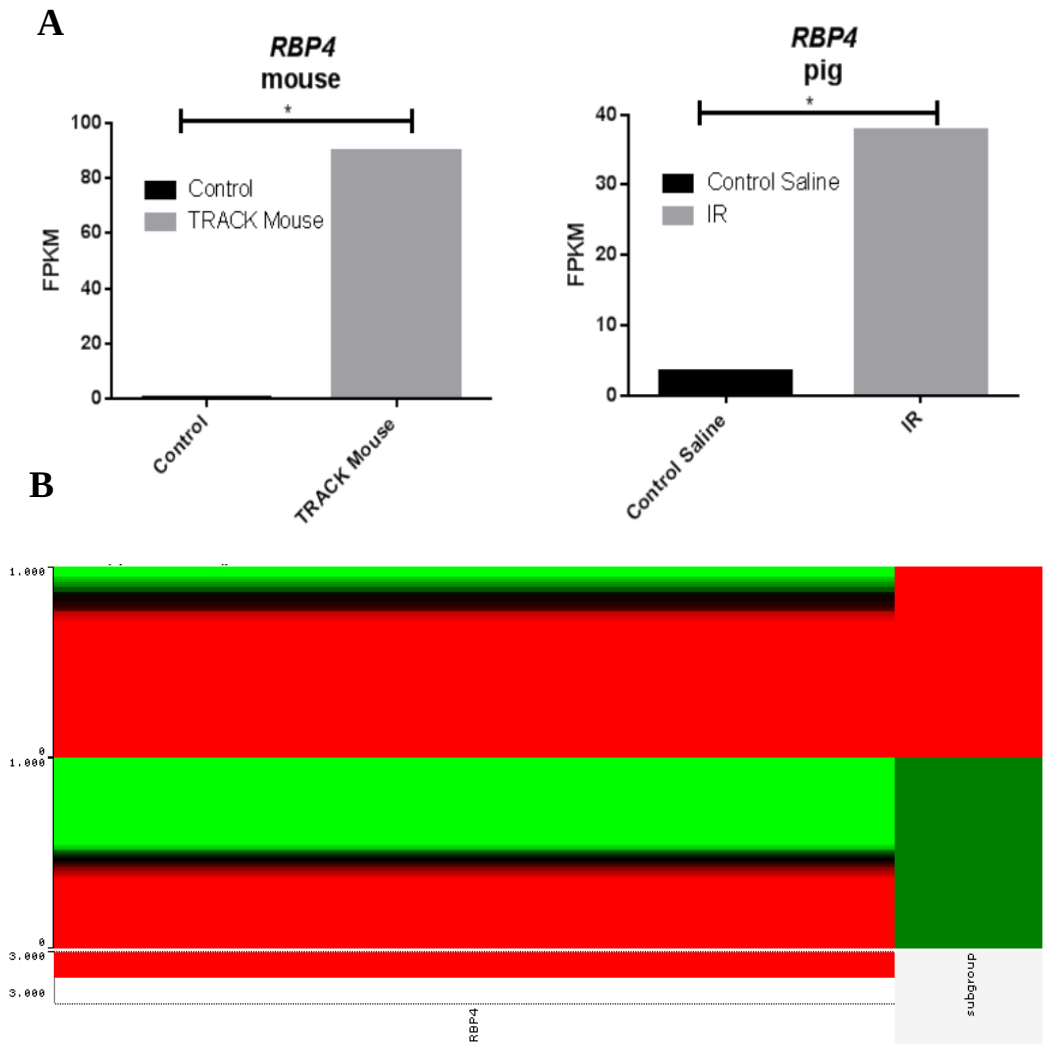


Figure 3.5: **(A)** Expression plot shows significant differential expression of the RBP4 gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of RBP4 gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that RBP4 is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal renal tissue (Red) as compared to matched tumour specimens (Green).

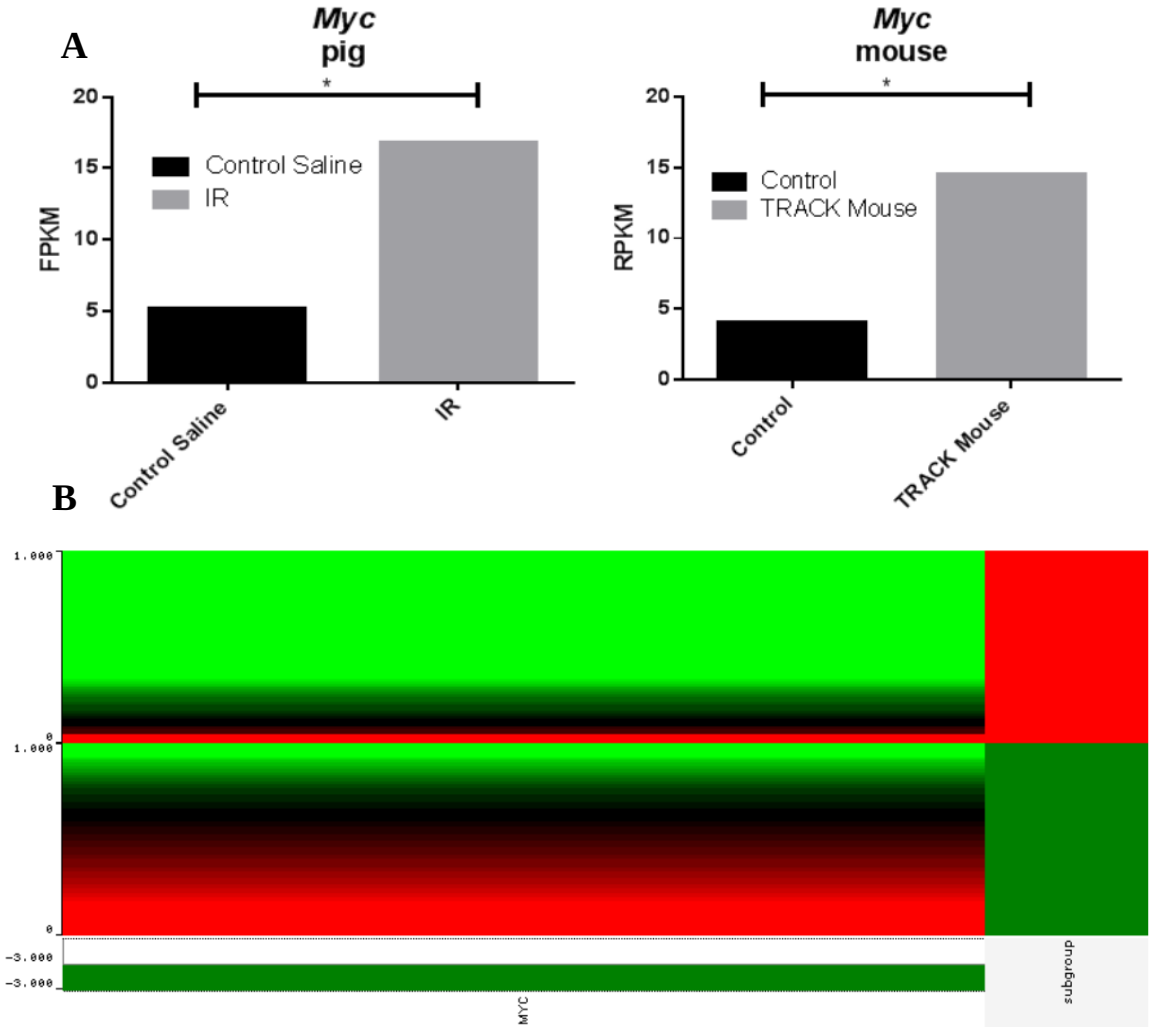


Figure 3.6: **(A)** Expression plot shows significant differential expression of the *Myc* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *Myc* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *Myc is* significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

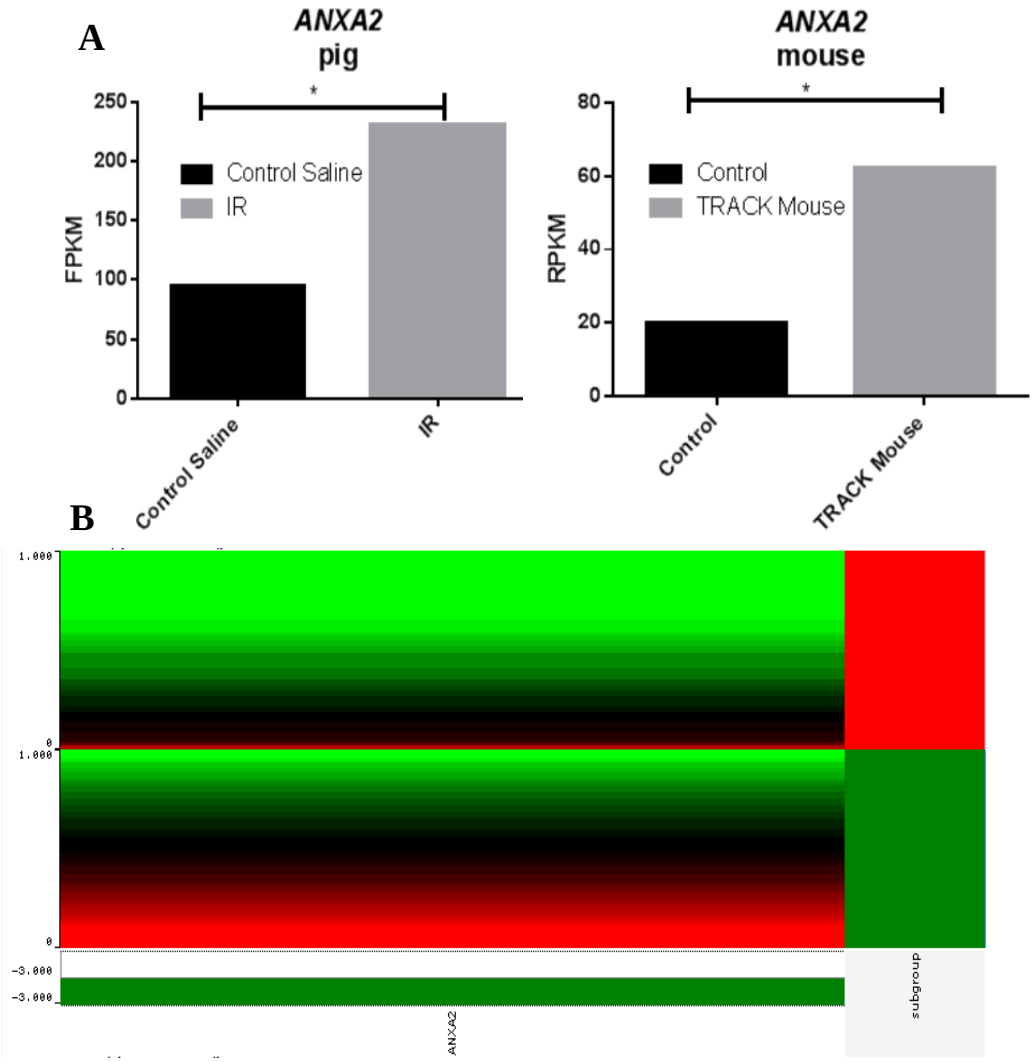


Figure 3.7: **(A)** Expression plot shows significant differential expression of the ANXA2 gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *ANXA2* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *ANXA2* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

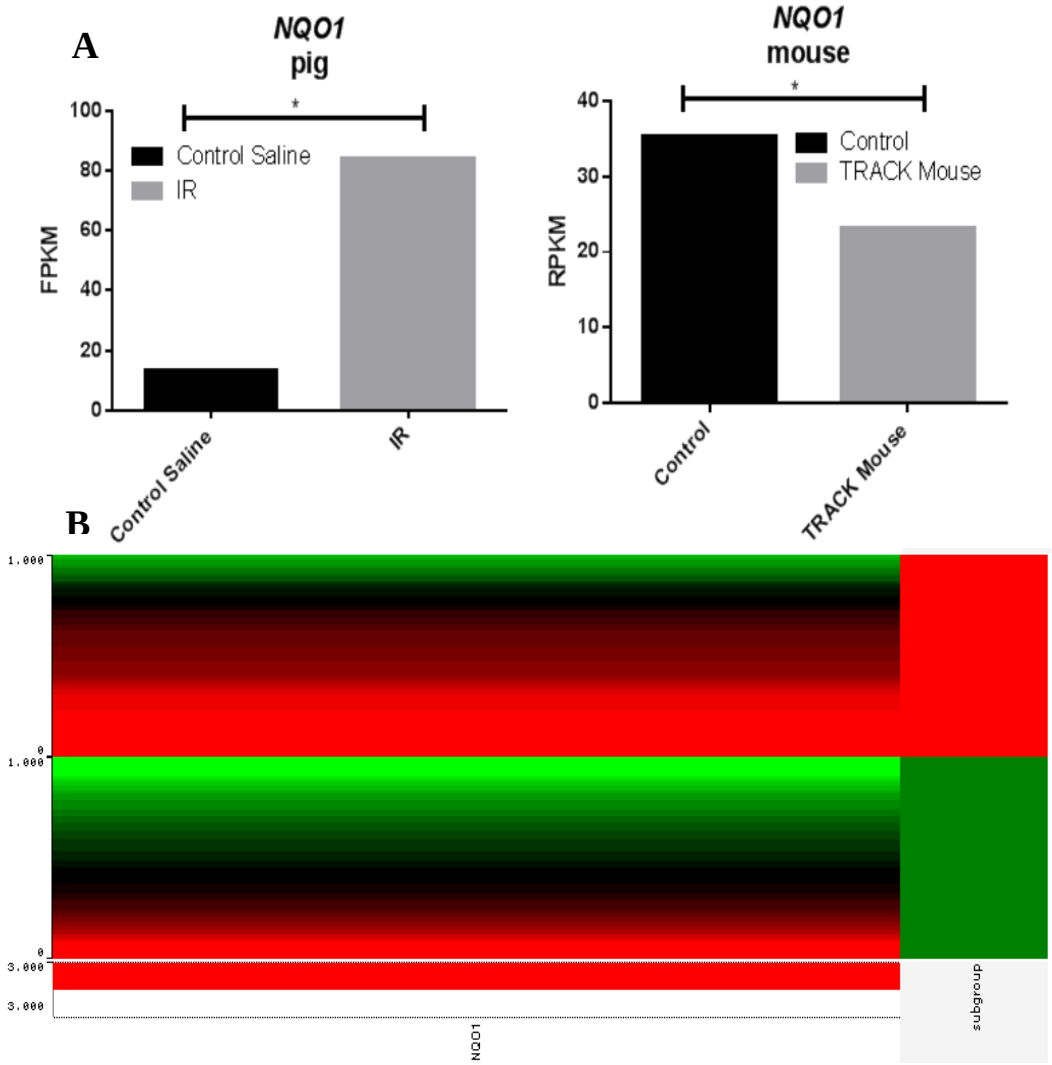


Figure 3.8; **(A)** Expression plot shows significant differential expression of the *NQO1* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *NQO1* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *NQO1* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

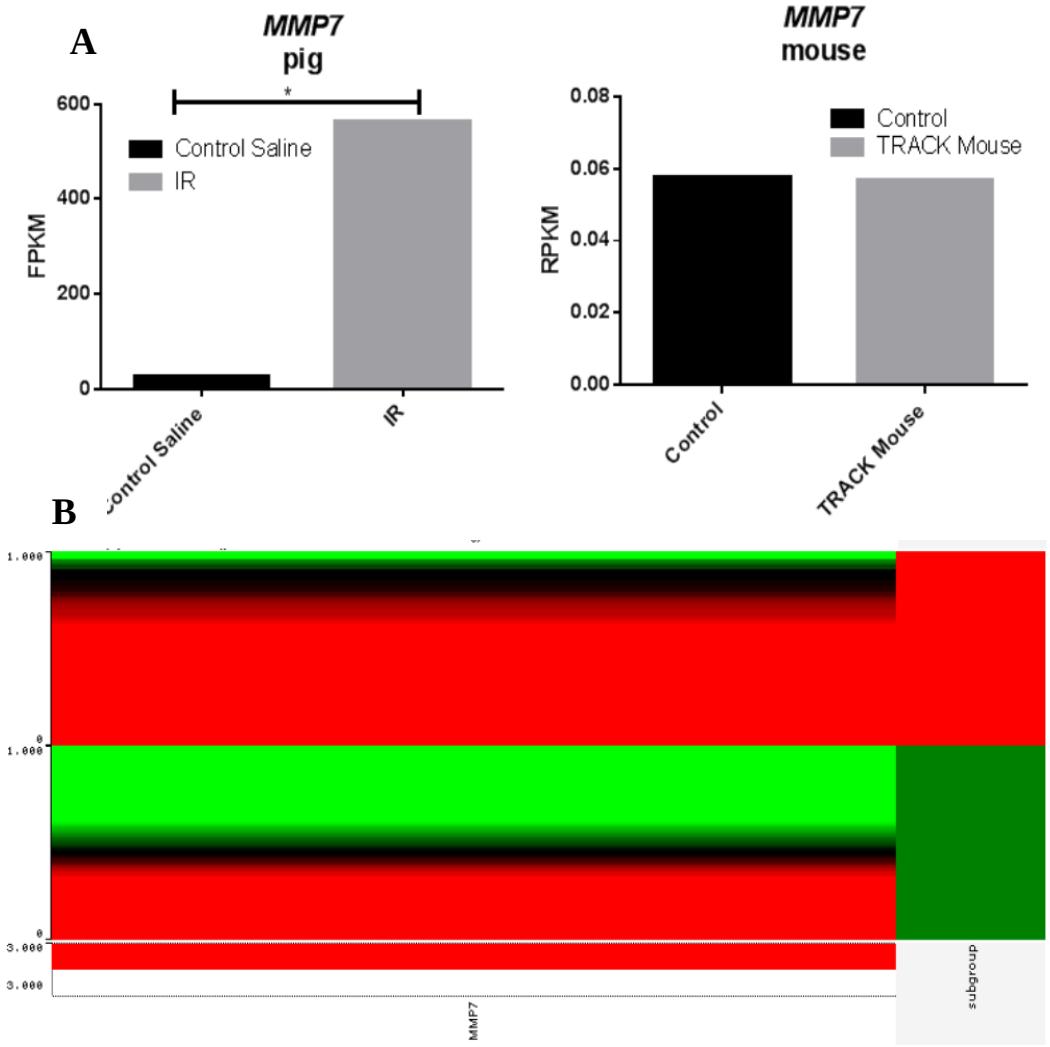


Figure 3.9: **(A)** Expression plot shows significant differential expression of the *MMP7* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *MMP7* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *MMP7* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

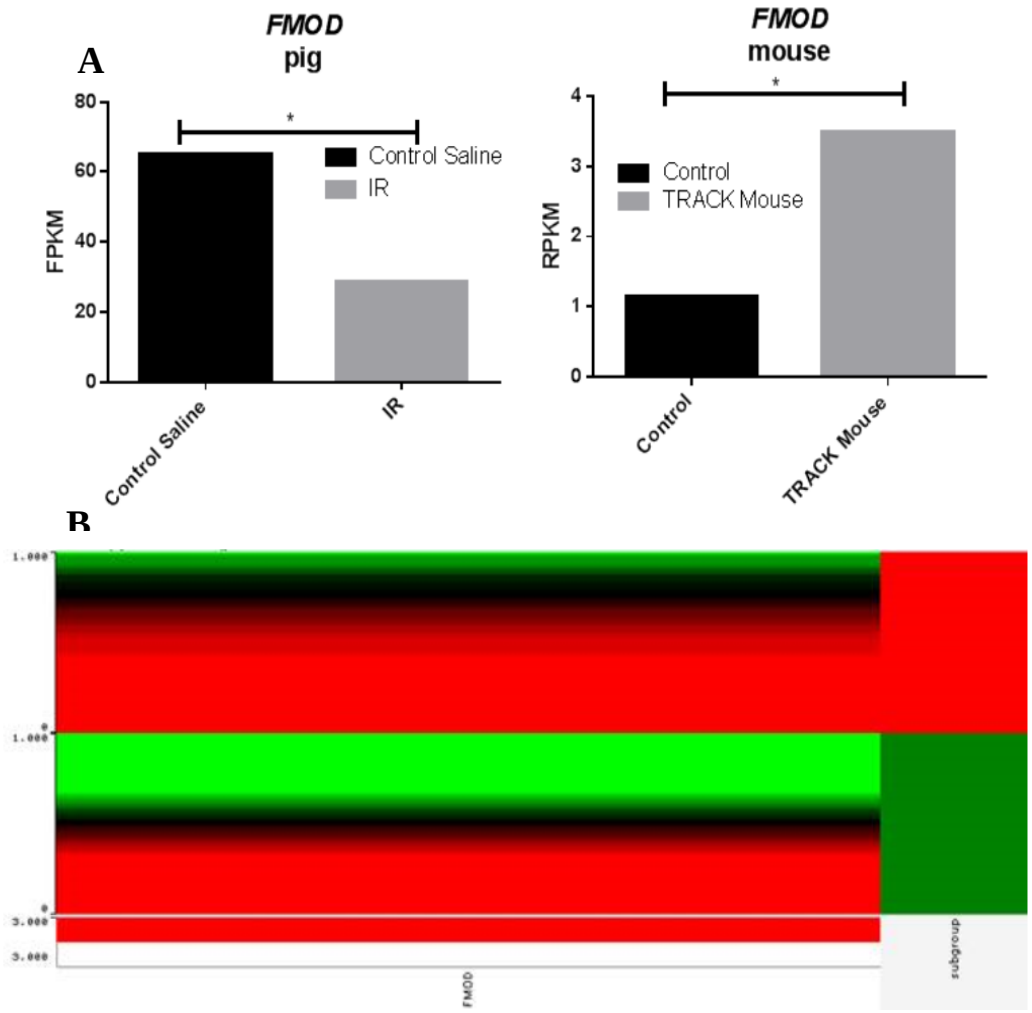


Figure 3.10: **(A)** Expression plot shows significant differential expression of the *FMOD* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *FMOD* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *FMOD* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

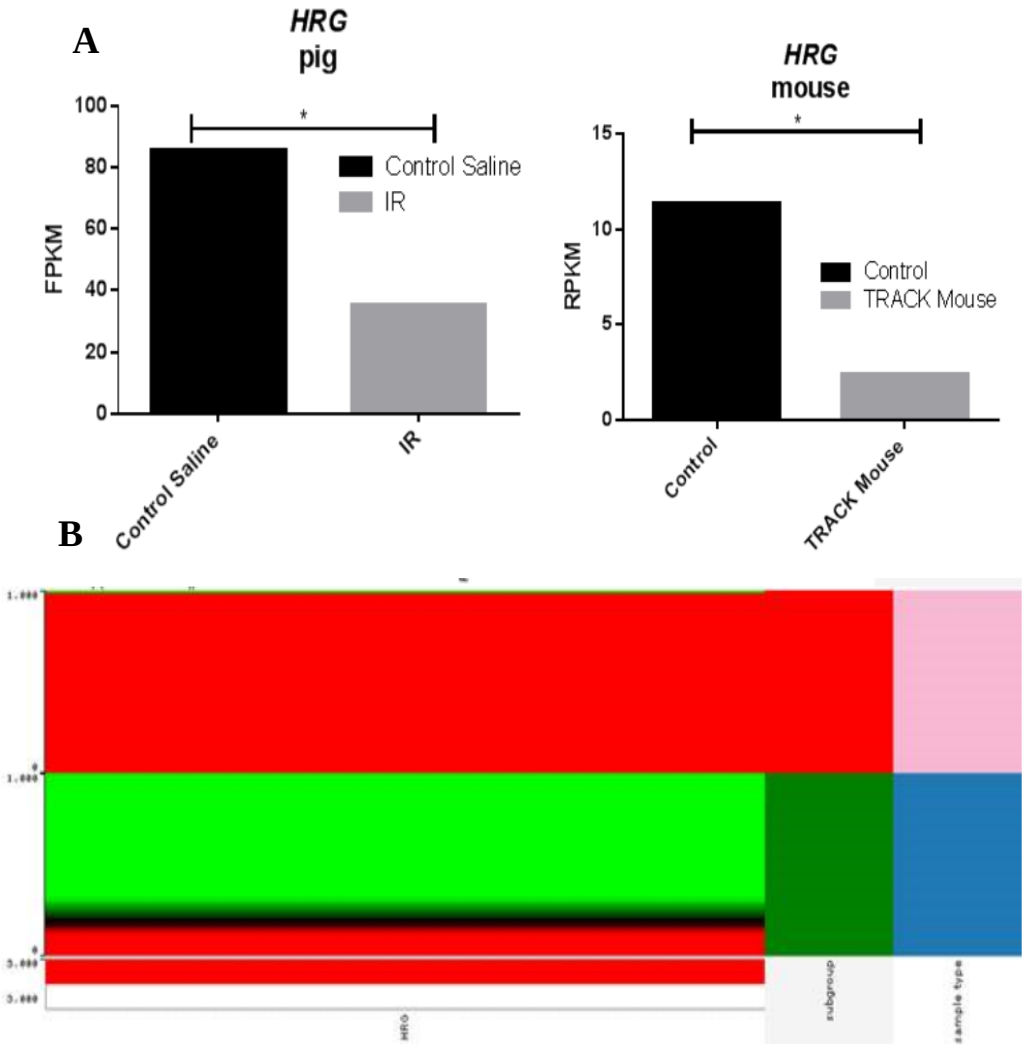


Figure 3.11: **(A)** Expression plot shows significant differential expression of the *HRG* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *HRG* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *HRG* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

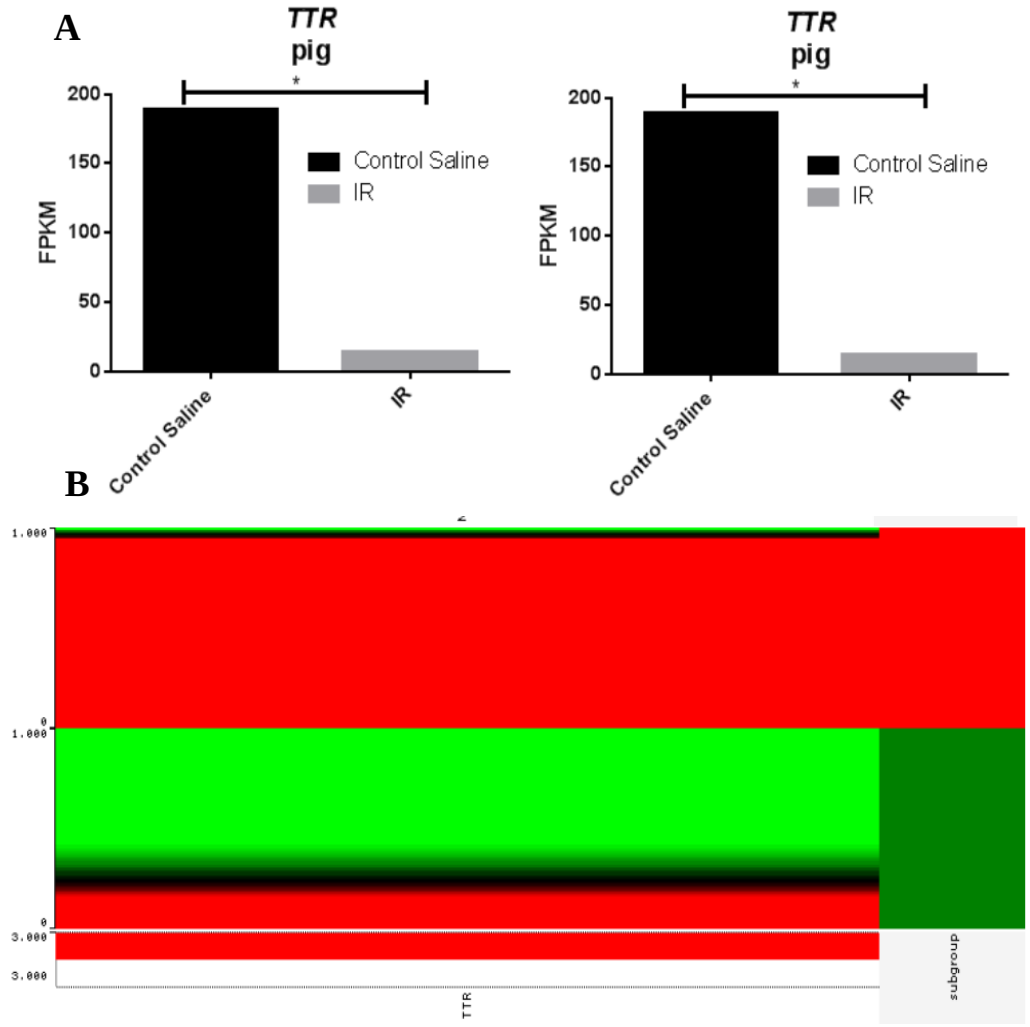


Figure 3.12: **(A)** Expression plot shows significant differential expression of the *TTR* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *TTR* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *TTR* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

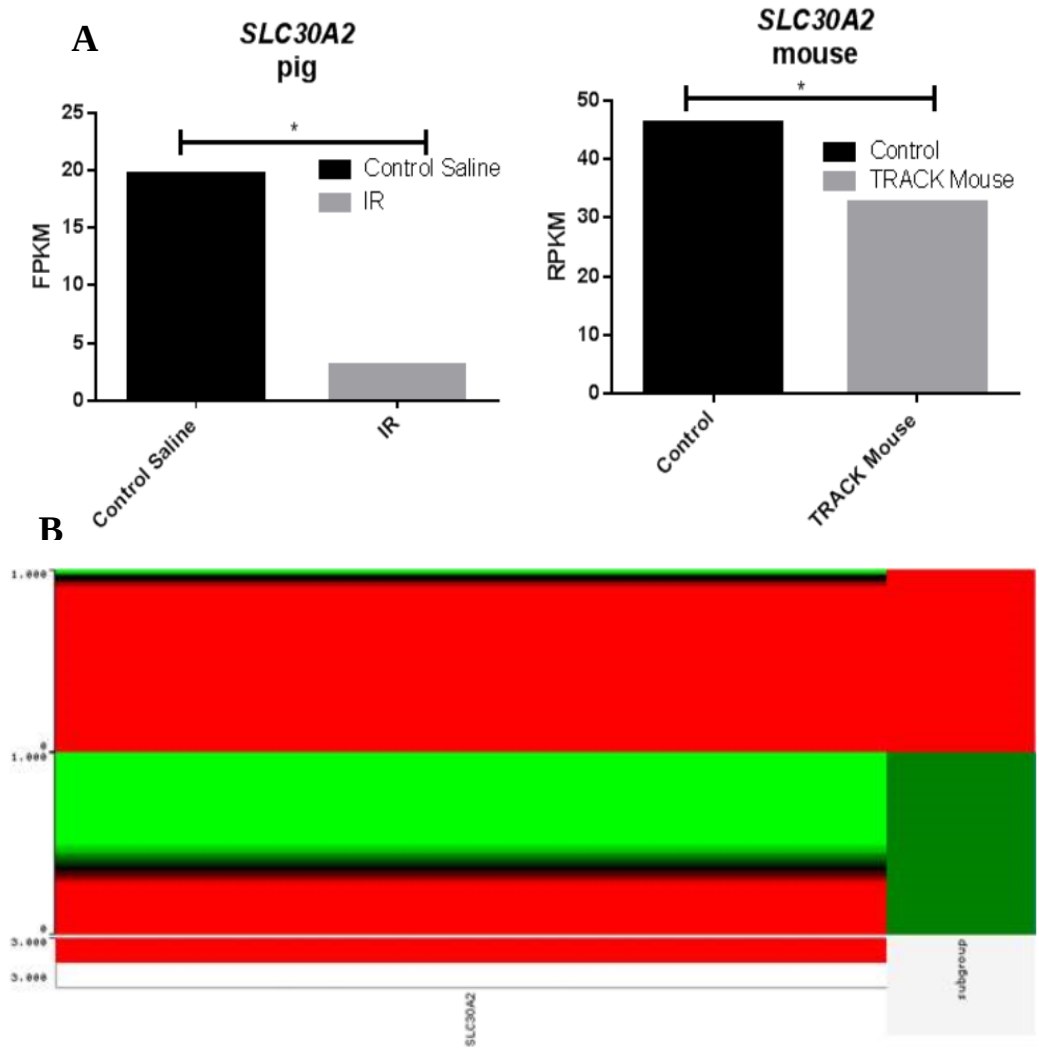


Figure 3.13: **(A)** Expression plot shows significant differential expression of the *SLC30A2* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *SLC30A2* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *SLC30A2* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

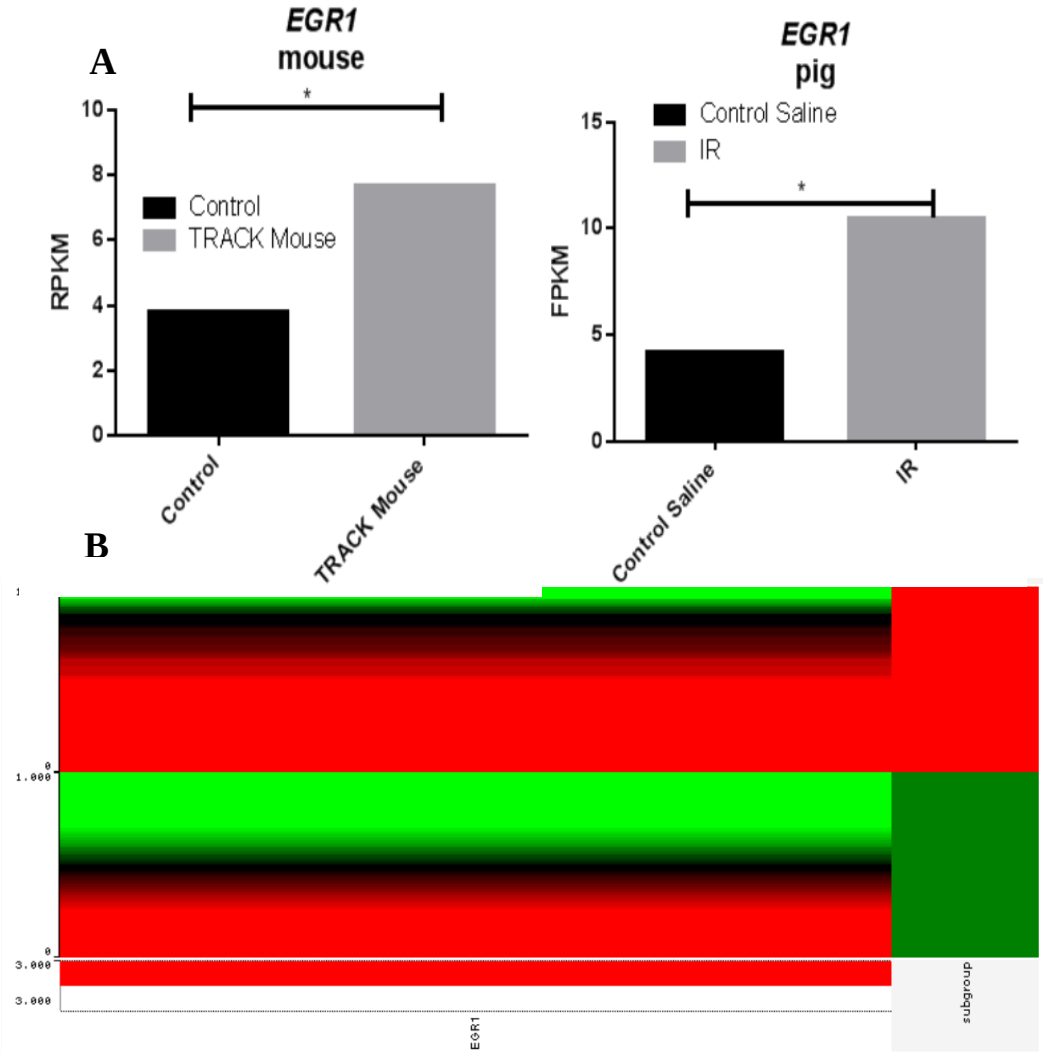


Figure 3.14: **(A)** Expression plot shows significant differential expression of the *EGR1* gene across control and ccRCC in mouse model and pig control as compared to AKI, as measured by RNA sequencing with Benjamin-Hochberg (BH) post-hoc correction ($p>0.05$). **(B)** Gene Expression analysis of *EGR1* gene on Cancer Genome Browser with human data in TCGA KIRC RNAseqV2 (N=606 patients). The heatmap shows that *EGR1* is significantly (t-test with Benjamini-Hochberg FDR post-hoc correction) more highly expressed in normal patients (Red) than tumour (Green).

3.3 Discussion

There is an urgent need to develop new methods to diagnose and treat both AKI and ccRCC. For this reason in the last decade new and improved models for AKI (Nagasaki et al., 2011; Bellomo et al., 2012; Singh et al., 2012; Skrypnyk et al., 2013; Ramesh and Ranganathan, 2014; Jang et al., 2015; Kate etal., 2016) and ccRCC (Rankin et al., 2006; Fu et al., 2011; Giraud, et al., 2011; Albers et al., 2013; Pritchett et al., 2015; Schokrpur et al., 2016) were developed. Ischemia reperfusion injury is the most frequent reason of AKI in both native and transplanted kidneys (IRI) (Liano and Pascual, 1996; Mehta et al., 2004; Devarajan, 2006; Jang et al., 2009; Bonventre and Yang, 2011; Giraud, et al., 2011; Basile et al., 2012; Malek and Nematbakhsh, 2015; Nielsen et al., 2017). It is established that the anatomical and metabolic characteristics of kidney determine the mechanism of IRI (Silva, 1990; Giraud, et al., 2011; Liu et al., 2012; Wei et al., 2014; Nielsen et al., 2017). Ischemia/reperfusion created resilient, extensive changes of metabolites in kidney cortex, kidney medulla, and plasma over time (Wei et al., 2014). Literature reported some metabolites that showed continuous or sustained decrease in kidney tissues in all reperfusion time points for example serine and threonine metabolism, N-methyldiethanolamine for glycine, glutamate and glutamine for glutamate metabolism, inosine for purine metabolism [(hypo) xanthine/inosine containing); betaine and Isobar: betaine aldehyde, pentose metabolism; nicotinamide for nicotinate and nicotinamide metabolism; and succinylcarnitine (Liu et al., 2012; Wei et al., 2014) whereas other metabolites viz 3-indoxyl sulfate significantly increases in AKI models and Patients (Zgoda-Pols et al., 2011; Wu et al., 2013; Uehara et al., 2014). The major research in this field is conducted on the smaller animals and the data obtained from these models is difficult to relate to human models (Lieberthal and

Nigam, 2000). Larger animals are considered appropriate models for the study of IRI especially for the transplantation studies. Pig kidney is structurally more closely associated with the humans and is considered an idealy model for such studies (Sampaio et al., 1998; Lieberthal and Nigam, 2000; Pereira-Sampaio et al., 2004; Helio et al., 2008).

In the present study the analysis of 2 x control_saline and 2 x IR (ischemia reperfusion) pig samples were done and compared these to the control (x3) and TRACK (x3) mice differential gene lists from cuffdiff. This has identified a cohort of 37 genes common to both disease processes. Several of these genes in the pig samples were validated. It was better to increase the number of samples of pig as well as human sample so the data will be more robust. Also only validated genes at mRNA levels, therefore need to validate at protein level too.

This project was focused on transcriptional changes not on metabolic changes in both conditions that was subjected to another related Masters project at the same time.

RNA sequencing was used to characterize the transcriptomes of (i) kidney specimens from pig AKI samples derived from ischemia reperfusion as a result of a 40 minute bilateral cross clamping of renal arteries followed by 48 hour reperfusion (David Gardner, personal communication) and (ii) kidney cancer specimens from the mouse TRACK model of ccRCC. This identified a key network of 37 differential genes common to both disease processes. The expression of these 37 genes were then analyzed using the cancer genome atlas KIRC (n=660) RNA sequencing dataset. The expression of *MYC, ANXA2* and *EGR1* were validated in both pig AKI and TRACK ccRCC by qRTCPR (N.Mongan, personal communication). In this study we report

the qRTPCR validation of additional genes including *RBP4, FMOD, NQO1, MMP7, SLC30A2, HRG, TTR* and *SLPI.*

SYBR-green qRTPCR analysis revealed that expression of *MYC, RBP4, ANAXA* genes but not *EGR1* were significantly increased in tumour (TRACK). In pig AKI samples, hydrolysis probe (TaqMan™) qRTPCR analysis of expression of *MYC, RBP4, FMOD, NQO1, MMP7* and *EGR1* were significantly higher relative to control samples, but expression of *ANXA2, SLC30A2, TTR, HRG* and *SLPI* were unchanged. Interestingly, of these genes, only expression of *MYC* and *ANXA2* are higher in human ccRCC samples relative to control non-malignant kidney in the TCGA KIRC RNAseqV2 database. One potential reason for this is that the non-malignant tissues included in the TCGA dataset are matched from patients with ccRCC. As the location of the source of the non-malignant tissue within the kidney relative to the ccRCC tumour is not available, it is possible that the well-establish local tumour "field effect", whereby malignant tissue can influence gene expression of adjacent grossly normal tissue so that it more resembles malignant tissue. This may mask differential gene expression effects between true normal tissue and cancer.

Also In the present study Genome browser tools were used that have its own limitations viz limited dimensions to show relationships among genomic features like coordinated expression of genes. The IGV and UCSC Cancer Genomics Browser Somehow overcome this problem by heatmap approach but it has its own limitation. For example it is tough to discriminate the false of copy-number alteration (CAN) among several genes is possible synergy or outcome of their location within

frequently amplified or Sorted chromosomic fragments. The function and biological relevance of these validated genes in kidney physiology and pathology is discussed below.

3.3.1 MYC

MYC is implicated in the tumourigenesis of different forms of human malignancies and affects the expression of wide-ranging human genes related to carcinogenesis (Patel et al., 2004). *MYC* expression has been shown to be important in control of G1/S transition in cell cycle progression of cancer cells. For example as breast cancer, osteosarcoma, lung carcinoma, lymphoblastoid, and menaloma cell lines (Prall et al., 1998, Pajic et al., 2000, Santoni-Rugiu et al., 2000, Qi et al., 2007, Wang et al., 2008). *MYC* play role as modulator of cell cycle progression by controling the expression of cell cycle regulators (Zajac-Kaye, 2001, Carroll et al., 2002, Qi et al., 2007). The *MYC* oncogene involves in different mechanism through that it contributes to tumourigenesis of many types of cancer (Felsher and Bishop, 1999a, Felsher and Bishop, 1999b, Jain et al., 2002, Arvanitis and Felsher, 2005). The regulation of proliferation and growth, protein and ribosomal biogenesis, changes in metabolism, lipid synthesis, and induction of angiogenesis are some of the mechanism showing MYC gene involvement (Dang, 1999, Giuriato et al., 2006, Rakhra et al., 2010, van Riggelen et al., 2010). MYC expression can promote angiogenesis by increasing the expression of VEGFA, which plays a role in tumour angiogenesis (Veikkola et al., 2000, Pelengaris et al., 2002). In ccRCC, Tang and colleagues (2009) demonstrated the activation of MYC-regulated pathways and its important roles in cell cycle progression, cellular proliferation and anchorage-independent growth of ccRCC cells. The MYC pathway is activated in most cases of human RCC (Tang et al., 2009), amplified in 5–10% of patients, over-expressed in 20% (Creighton CJ, 2013) and is

associated with a hereditary RCC syndrome (Drabkin et al., 1985) suggesting a causal role in the pathogenesis.

3.3.2 ANAX2

Annexin A2 (*ANXA2*) coordinates multiple biologic processes and is associated with cancer progression. The structure of *ANXA2* affects its cellular localization and function. Intracellular *ANXA2* has been reported to play roles in exocytosis (Sarafian et al., 1991), endocytosis (Emans et al., 1993, Morel and Gruenberg, 2007) and membrane trafficking (Babiychuk and Draeger, 2000). Furthermore, *ANXA2* is essential for lipid raft formation and signal transduction through its interaction with CD44, a cell receptor for hyaluronic acid that is richly expressed in lipid rafts (Oliferenko et al., 1999) Interestingly, Martin et al. in their recent findings suggest that *ANXA2* serves as a ligand for C1q on apoptotic cells (Martin et al., 2012). *ANXA2* degradation is correlated with cellular apoptosis induced by p53-mediated pathways (Huang et al., 2005). For better understanding of molecular mechanism of ccRCC, require the identification of tumour-associated genes and construction of full-length enriched cDNA libraries of ccRCC and normal kidney tissues.

Increased expression of *ANXA2* is frequently observed in many cancer cell types. Overall, *ANXA2* is over-expressed in acute lymphoblastic leukemia (ALL) (Spijkers-Hagelstein et al., 2013), acute promyelocytic leukemia (APL) (Menell et al., 1999), breast cancer (Sharma et al., 2006a), colorectal carcinoma (CRC) (Emoto et al., 2001b), gastric cancer (Emoto et al., 2001b) glioma (Roseman et al., 1994), hepatocellular carcinoma (HCC) (Mohammad et al., 2008), lung cancer (Sharma et al., 2006b, Wang et al., 2012a, Jia et al., 2013), multiple myeloma (MM) (Bao et al., 2009), oral squamous cell carcinoma (OSCC) (Rodrigo et al., 2011), and pancreatic

cancer (Vishwanatha et al., 1993, Esposito et al., 2006). In addition to metastasis and invasion, previous studies have shown that *ANXA2* is involved in tumour cell proliferation (Matsunaga et al., 2004, Bao et al., 2009, Braden et al., 2009).

3.3.3 RBP4

Retinol binding protein (Ad-hoc working group of Erbp) is a 21-kDa protein which is synthesized in the liver and involve in transporting vitamin A from the liver to other tissues. The glomerulus of kidney filtered it and further it catabolized and reabsorbed by the PCT. Bernard et al. found urinary *RBP* that considered as a highly sensitive indicator of renal tubule dysfunction as it precedes urinary NAG elevation in AKI patients from various etiologies (Bernard et al., 1987). Furthermore, according to Roberts et al., AKI in infants following birth asphyxia, the increased *RBP* levels in the first two days of life were predictive of clinically significant. In child this setting where reading of serum creatinine is predominantly challenging as it shows the maternal serum concentration to a noteworthy level (Roberts et al., 1990). However its potential significance in AKI and ccRCC remains poorly understood.

3.3.4 NQO1

NAD (P) H: quinone oxidoreductase (*NQO1*) is a ubiquitous cytosolic enzyme that comprises an obligatory two-electron reductase and catalyzes the reduction of quinone substrates. *NQO1* gene expression is regulated by the ARE (antioxidant response element) in various tissues both in normal condition and during oxidative stress conditions (Nioi and Hayes, 2004). *NQO1* is an inducible antioxidant enzyme. Its abundance is increased several orders of magnitude in response to the antioxidant Keap1/Nrf2/ARE pathway. Although a reduced or absent *NQO1* activity has been correlated with increased risk for development of human cancers (Lin et al., 1999,

Traver et al., 1992), numerous studies specified upregulation of *NQO1* in a number of cancers such as breast cancer, pancreatic cancer, cervical cancer, colorectal cancer, uterine melanoma, cholangiocarcinoma and lung cancer (Ma et al., 2014, Yang et al., 2014). The upregulation of *NQO1* specifically in colorectal, breast and cervical cancers, found to be associated with the late clinical stage of the disease, poor differentiation and lymph node metastasis (Ma et al., 2014, Yang et al., 2014). When impact of expression levels of *NQO1* was compared in patients with breast and cervical cancer, they show reduced disease-free Survival (DFS) and 5-year overall survival (OS) rates in highly expressing *NQO1* patients than low expressing patients (Ma et al., 2014, Yang et al., 2014). Apart from that *NQO1* activity can be noticed in many cancers that depict it is significantly higher than that in adjacent normal tissues (Terai et al., 2009, Cresteil and Jaiswal, 1991, Beall et al., 1995, Siegel et al., 1998).

3.3.5 HRG

Histidine-rich glycoprotein (*HRG*) is an abundant plasma glycoprotein that regulates several important biological processes. HRG has a multi-domain structure and it interacts with many ligands. Several cases associated with sites of tissue injury or tumour growth have been observed where the histidine-rich region of the molecule increases ligand binding that subsequently interact with Zn (2+) or exposure to low pH conditions (Jones et al., 2005). It has shown that *HRG* can modulate angiogenesis by a number of literatures (Guan et al., 2004, Brown and Parish, 1994, Simantov et al., 2001, Juarez et al., 2002, Donate et al., 2004, Olsson et al., 2004). Furthermore, its characteristic ability in regulation of many other physiological processes such as cell adhesion and migration (Hajjar et al., 1987, Chang et al., 1992, Lamb-Wharton and Morgan, 1993, Olsen et al., 1996, Mori et al., 2003), fibrinolysis and coagulation (Lijnen et al., 1983, Leung et al., 1989, Chang et al., 1994, Gorgani et al., 1996,

Gorgani et al., 2002, Borza and Morgan, 1997), complement activation, immune complex clearance and phagocytosis of apoptotic cells (Chang et al., 1992, Gorgani et al., 1996, Gorgani et al., 1997, Gorgani et al., 1999) have been shown in studies.

3.3.6 MMP7

MMP7 belong to matrix metalloproteinase (MMP) family is a major family of ECM-degrading enzymes and well known for its role in cancer invasion and metastasis (Nagase and Woessner, 1999, DeClerck et al., 2004). Tumour aggressiveness is influenced by many factors but destruction of the extracellular matrix (ECM) is one of the major early steps in a number of malignant tumours. Till date the complex role of matrix metalloproteinase-7 (*MMP-7*) or matrilysin 1 in cancer is poorly understood. Literature suggests *MMP7* involvement in cell proliferation and angiogenesis in various cancers in addition to its invasive function (Di Nezza et al., 2002, Egeblad and Werb, 2002, Jiang et al., 2006). These features directing several researchers to considering pathological significance of *MMP7* in cancer as well as the role of *MMP* inhibitors as anti-tumour cure of different cancers. Patraki and Cardillo (2007) investigated differential expression of *MMP7* and reported that it expressed variably in peritumoural normal areas than in benign epithelial neoplasias (renal papillary and oncocytomas) and RCC carcinomas. When compared in different subtypes of cancer it show highest immunopositive reaction in chromophobe RCC subtypes, followed by conventional clear-cell and chromophilic-papillary RCC and the lowest levels in spindle-cell and collecting-duct RCCs (more aggressive RCC). They also observed that *MMP7* reached highest levels in high-grade and high-stage RCCs (Patraki and Cardillo, 2007). This advocates MMP7 has significant role in the development and progression of renal cancer. Differentia expression of *MMP7* imitate more aggressive behavior along with malignant phenotype of ccRCCs in the various histological

RCC subtypes In addition, Lu et al., compares expression of expression of *MMP7* in ccRCC tissues and depicted significantly higher expression compared with that in the control group. The expression of *MMP7* in the ccRCC tissues was significantly correlated with the pathological grade and clinical stage (Lu et al., 2013).

3.4 Conclusion

Of the 37 genes identified common to AKI and ccRCC, the differential expression of *MYC* and *ANXA2* suggest a mechanistic link between these AKI and ccRCC. These findings are consistent with previous reports describing the activation of *MYC* pathway in hereditary RCC (Drabkin et al., 1985, Tang et al., 2009, Creighton CJ, 2013). *MYC* is one of the oncogenes which are seen most often to be mutated and expressed at very high levels in human renal cell carcinoma (RCC). However, the role of *MYC* or any other known oncogenes in maintaining or initiating kidney cancer is still poorly understood (Furge et al., 2007, Gordan et al., 2008, Beroukhim et al., 2009, Tang et al., 2009, Yamamura et al., 2012, Liu et al., 2013). Earlier studies have suggested that inactivation of *MYC* gene can bring about arrest of cell cycle, differentiation and apoptosis. Thus inactivation of *MYC* gene can lead to regression of many types of tumours (Arvanitis and Felsher, 2006, Watt et al., 2008). It has also been established that expression of *MYC* play an important role in progression through G1/S phase of cell cycle in different types of cancers such as osteosarcoma, breast cancer, lung carcinoma, lymphoblastoid, and menaloma cell lines (Prall et al., 1998, Pajic et al., 2000, Santoni-Rugiu et al., 2000, Qi et al., 2007, Wang et al., 2008). MYC controls the progression through the cell cycle by regulating the expression of cell cycle regulators (Zajac-Kaye, 2001, Carroll et al., 2002, Qi et al., 2007). Tumours induced by *MYC* exhibit phenotypic characteristics of very aggressive subform of renal cell collecting-duct carcinoma (Liu et al., 2013). The upregulation of *MYC* was

validated in ccRCC tissues and cell lines. Abnormal proliferation, anchorage independent growth and cell cycle arrested in the G0/G1 phase in ccRCC cells was significantly inhibited when *MYC* gene has been knockdown by *MYC*-specific siRNA (Tang et al., 2009). Differential expression of *MYC* gene is also observed in different types of cancers such as glioblastoma, colon cancer and breast cancer and plays critical roles in tumourigenesis (Nesbit et al., 1999, Pelengaris et al., 2002). *MYC* function as a transcription factor which binds to the CACGTG sequence (E-box motif) of DNA and can affect the expression of large number of human genes which play role in the development of cell carcinogenesis viz cell cycle progression, cell growth, metabolism, apoptosis, and angiogenesis (Adhikary and Eilers, 2005, Dang et al., 2006, Knoepfler, 2007). In this study, up-regulation of *MYC* expression was found in both AKI and in a mouse model of ccRCC and in human ccRCC. Kozma et al reported *MYC* amplification in ~8% of ccRCC (He et al., 1998, Vita and Henriksson, 2006), however, Fan et al. showed that 17-18 percent of ccRCC tissues exhibit over-expression of *MYC* gene (Fan et al., 2005). In ccRCC tissue it is believed that *MYC* activation can take through mechanism(s) other than the mechanism involved in the *MYC* amplification pathway. However, in majority of RCC cases the MYC pathway is activated (Tang et al., 2009), and it may involve genomic amplification in 5-10% patients, over-expression in about 20 % patients (Creighton CJ, 2013), and it is also linked with the hereditary RCC syndrome (Drabkin et al., 1985).

ANXA2 is another gene which is expressed appreciably in the AKI, the TRACK mouse model and ccRCC patients. It is also known as p36 or calpactin I, lipocortin II, annexin II, placental anticoagulant protein IV or chromobindin VIII (Hajjar and Krishnan, 1999) and produces a protein of 36 kDa (Hajjar and Krishnan, 1999). The expression of gene produces a protein annexin II which is associated with various

physiological activities such as anti-inflammatory, endocytosis, exocytosis and anticoagulation (Sarafian et al., 1991, Emans et al., 1993, Hajjar et al., 1998, Morel and Gruenberg, 2007), regulation of trafficking across the membrane (Babiychuk and Draeger, 2000) signal transduction, differentiation and apoptosis (Hanahan and Weinberg, 2011b, Sharma and Sharma, 2007, Bao et al., 2009, Lokman et al., 2011), cell proliferation (Lokman et al., 2011), phagocytosis (Fan et al., 2004), fibrinolysis and anticoagulation (Choi et al., 2001, Ling et al., 2004), angiogenesis (Ling et al., 2004), and cell metastasis(Mai et al., 2000, Sharma et al., 2006a). Knockdown of *ANXA2* is knocked results in inhibition of cell division and proliferation (Chiang et al., 1999).

Malignant tumours are characterized by unregulated growth of the cells. It has been established that *ANXA2* play a critical role in the synthesis of DNA, proliferation of cells and thus is responsible for cell cycle regulation (Eberhard et al., 2001). It has been observed that the *ANXA2* is over-expressed in many of the cancers like breast cancer (Sharma et al., 2006), APL (Menell et al., 1999), gastric cancer (Emoto et al., 2001a), acute lymphoblastic leukemia (ALL) (Spijkers-Hagelstein et al., 2013), colorectal carcinoma (CRC; (Emoto et al., 2001a), oral squamous cell carcinoma (OSCC) (Rodrigo et al., 2011), glioma (Roseman et al., 1994), lung cancer (Sharma et al., 2006a, Wang et al., 2012a, Jia et al., 2013), multiple myeloma (MM) (Bao et al., 2009), hepatocellular carcinoma (HCC, (Mohammad et al., 2008), ccRCC (Zhong et al., 2009) and pancreatic cancer (Vishwanatha et al., 1993, Esposito et al., 2006). ANXA2 is also associated cancer invasion and metastasis. A research work reported by Ohno et al. revealed that in comparison to the normal renal cortex the expression of *ANXA2* is up-regulated in primary clear cell renal carcinoma cells (ccRCCs). Primary tissue of ccRCC and metastatic tumours were found to be positive for *ANXA2*

in 47.4% and 87.5% of cases respectively (Ohno et al., 2009). Expression of *ANXA2* in primary tissue is however it is associated with the progression to the later stages. The expression of *ANXA2* gene was approximately 1.64 times enhanced in the tissue of RCC tumours when compared to the normal tissue which implicates that over-expression is positively correlated with the development of RCC (Hirano et al., 2006).

Collectively the results reported in this chapter is the first mechanistic evidence suggesting a link between AKI and ccRCC. Further work is required to substantiate this potential link in AKI and ccRCC patients.

Chapter 4: Conclusions and Future directions

4.1 Conclusion

The important role of hypoxia in AKI and ccRCC provided the rationale for this study. As noted earlier altered cell metabolism is characteristic of ccRCC and that is the outcome of increased *HIF*-responsive genes expression involved in glucose uptake, enhanced glycolytic metabolism, and decreased mitochondrial respiration. *NDUFA4L2* is one of the most significantly expressed genes in ccRCC plays a major role in altering cellular metabolism. TRACK mice reveal increased expression of *HIF-1α* targeted genes responsible for shift in metabolism. The present study investigated whether *HIF-1α* over-expression increased genomic instability, as evidenced by alterations in transcription, splicing, transcript fusions or induction of SNVs. However the RNA sequencing analyzed here did not identify such transcriptional alterations in the TRACK mice, suggesting *HIF-1α* over-expression does not affect significantly alter these features in TRACK mice. This analysis did identify unique SNVs in the TRACK ccRCC model relative to wild-type control mice. Multiple variants in TRACK mice were identified including an intronic variant in the *NDUFA4L2* locus predicted to alter *NDUFA4L2* splicing and a coding variant in the *CES3/Ces1d* gene. Further analysis showed the prevalence of mutations in these genes in human ccRCC genomic data. A novel *CES3* gene mutation was identified in mice over-expressing *HIF-1α* .*CES3* (carboxylesterase 3) gene is an orthologue of human *Ces1d*, also known triglyceride hydrolase, is involved in lipolysis by hydrolyzing stored triglycerides into fatty acids and facilitate the uptake and incorporation of nutrients into biomass that suggesting the potential functional significance of *CES3* the identified variants in ccRCC.

In the present study only *CES1d/CES3* is altered by coding-mutation and also showing splicing SNVs. Other ccRCC relevant genes including *HIF-1α, HIF-2α, VHL, NDUFA4L2, VEGFA* showed no coding alterations. Our mutation analysis identified variants predicted to alter splicing. Consistent with this, Cuffdiff and Sashimi plot analyses shows that *NDUFA4L2, Hilpda, Zbtb38, Cdc58, Plekhm2* and *Aldoa* splicing was affected. This may indicate a known limitation of RNAseq in the identification of mutations affecting splicing junctions. The present study also sought to identify *HIF-1α* over-expression-induced fusion transcripts. Again, no oncogenic fusion transcripts were identified in these samples. This supports the conclusion that *HIF-1α* over-expression does not appear to be pro-mutagenic.

The final chapter of the present study attempted to establish mechanistic links between AKI and kidney cancer. Thirty seven genes were found commonly differentially expressed by both the disease AKI and cancer, out of which 24 genes were commonly up or down regulated in both disease process. This suggests that these genes may contribute to pathogenic processes in both AKI and ccRCC. Several genes of interest like *MYC, ANXA2, EGR1, RBP4, FMOD, NQO1, MMP7, SLC30A2, HRG, TTR* and *SLPI* were validated by TaqMan PCR in AKI and TRACK kidney specimens. Present study, the validation is restricted to mRNA levels. The *MYC* oncogene is most frequently go under mutation and is overexpressed in human renal cell carcinoma (RCC). Recent report has illustrated that *ANXA2* is a RNA-binding protein which interacts with the nuclear oncogene mRNA along with *c-myc* and results in an increase in c-Myc protein (Filipenko et al., 2004). Therefore, the high expression of *ANXA2* gene may contribute to ccRCC tumourigenesis by increasing the c-Myc protein level. This is the first mechanistic evidence supporting a predisposing link between AKI and ccRCC.

4.2 Future Directions

Our result also show the potential role of a novel gene *CES3* mutation in renal clear cell carcinoma which is involved in lipid metabolism. To date the impact of nutrition on kidney disease progression is under-investigated. Therefore, one of the future directive works could establish the relationship between diet and the risk of RCC. Second future work is validation of gene of interest in AKI and cancer tissue by immunohistochemistry (IHC). IHC is a method that visualizes protein localization in situ in tissue sections using antibodies. This will represent a robust validation of the mRNA analyses described here and is underway.

The absence of appropriate transgenic animal models of renal cell carcinomas (RCCs) has made it difficult to identify and test new therapies for this disease. Only two genes were overexpressed in AKI, TRACK and in human tumours, whereas several other genes show high expression in cancer model. This may suggest that the TRACK model does not fully recapitulate the human tumour. Indeed one limitation to the TRACK model is its failure to metastasize. For this reason it will be necessary to develop a second generation TRACK model potential harboring additional genetic defects to develop a model of more aggressive disease.

Finally, the potential mechanistic links between AKI and ccRCC was established that will need to be substantiated in both retrospective and prospective longitudinal health studies. In future validation will be done both in pig, mouse and human samples. Collectively our data has been identified a common differential gene network implicated both in AKI and Kidney cancers. It is necessary to test translates or significance of expression of these genes in human tissue sample from patient with AKI and RCC. In addition it will possible to test this link by examining retrospective

of Clinical record and Co-relating incidence of AKI and ccRCC. Such knowledge is required to prove that AKI can increase risk of ccRCC in patients.

References

ABEYTA, M. J., CLARK, A. T., RODRIGUEZ, R. T., BODNAR, M. S., PERA, R. A. R. & FIRPO, M. T. 2004.Unique gene expression signatures of independently-derived human embryonic stem cell lines. Human Mol Gen, 13, 601-608.

ABRASS, C. K. 2004a. Cellular lipid metabolism and the role of lipids in progressive renal disease. *Am J Nephrol,* 24**,** 46-53.

ABRASS, C. K. 2004b. Overview: obesity: what does it have to do with kidney disease? *J Am Soc Nephrol,* 15**,** 2768-72.

AD-HOC WORKING GROUP OF ERBP, F. D. L. M. C. A. F. D. V. R. J. L. V. B. W. 2012. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. *Nephrol Dial Transplant.,* 27**,** 4263-72.

ADDLA, S. K., BROWN, M. D., HART, C. A., RAMANI, V. A. & CLARKE, N. W. 2008. Characterization of the Hoechst 33342 side population from normal and malignant human renal epithelial cells. *Am J Physiol Renal Physiol,* 295**,** F680-7.

ADHIKARY, S. & EILERS, M. 2005. Transcriptional regulation and transformation by Myc proteins. *Nat Rev Mol Cell Biol,* 6**,** 635-45.

ADLI, M., PARLAK1, M., LI, Y. & EL-DAHR, S. 2015. Epigenetic States of Nephron Progenitors and Epithelial Differentiation. J Cell Biochem, 116 (6), 893–902.

AHMED T. A. 2015. Pharmacokinetics of Drugs Following IV Bolus, IV Infusion, and Oral Administration. Basic Pharmaco Conc and Some Clin App, 53-98

AL-AWQATI, Q. & OLIVER, J. A. 2002. Stem cells in the kidney. *Kidney Int,* 61**,** 387-95.

AL-AYNATI, M., CHEN, V., SALAMA, S., SHUHAIBAR, H., TRELEAVEN, D. & VINCIC, L. 2003. Interobserver and intraobserver variability using the Fuhrman grading system for renal cell carcinoma. *Archives of Pathology and Laboratory Medicine,* 127**,** 593-596.

ALBERS, J., RAJSKI, M., SCHONENBERGER, D., HARLANDER, S., SCHRAML , P., TEICHMAN, VON A., GEORGIEV, S., WILD, P. J., MOCH, H., KREK, W. & FREW, I. J. 2013. Combined mutation of Vhl and Trp53 causes renal cysts and tumours in mice. *EMBO. Mol. Med,* 5, 949–964

ALISON, M. R., POULSOM, R. & FORBES, S. J. 2001. Update on hepatic stem cells. *Liver,* 21**,** 367-73.

ALLORY, Y., CULINE, S. & DE LA TAILLE, A. 2011. Kidney cancer pathology in the new context of targeted therapy.

AMELLEM, O., STOKKE, T., SANDVIK, J. A., SMEDSHAMMER, L. & PETTERSEN, E. O. 1997. Hypoxia-induced apoptosis in human cells with normal p53 status and function, without any alteration in the nuclear protein level. *Exp Cell Res,* 232**,** 361-70.

AN, W. G., KANEKAL, M., SIMON, M. C., MALTEPE, E., BLAGOSKLONNY, M. V. & NECKERS, L. M. 1998. Stabilization of wild-type p53 by hypoxia-inducible factor 1alpha. *Nature,* 392**,** 405-8.

ANDREOLI, S. P. 2009. Acute kidney injury in children. *Pediatr Nephrol,* 24**,** 253-63.

ANDREUCCI, M., MICHAEL, A., KRAMERS, C., PARK, K. M., CHEN, A., MATTHAEUS, T., ALESSANDRINI, A., HAQ, S., FORCE, T. & BONVENTRE, J. V. 2003. Renal ischemia/reperfusion and ATP depletion/repletion in LLC-PK(1) cells result in phosphorylation of FKHR and FKHRL1. *Kidney Int,* 64**,** 1189-98.

ANDREWS, D. W., SCOTT, C. B., SPERDUTO, P. W., FLANDERS, A. E., GASPAR, L. E., SCHELL, M. C., WERNER-WASIK, M., DEMAS, W., RYU, J., BAHARY, J. P., SOUHAMI, L., ROTMAN, M., MEHTA, M. P. & CURRAN, W. J. 2004. Whole brain radiation therapy with or without stereotactic radiosurgery boost for patients with one to three brain metastases: Phase III results of the RTOG 9508 randomised trial. *Lancet,* 363**,** 1665-1672.

ANGELOTTI, M. L., RONCONI, E., BALLERINI, L., PEIRED, A., MAZZINGHI, B., SAGRINATI, C., PARENTE, E., GACCI, M., CARINI, M., ROTONDI, M., FOGO, A. B., LAZZERI, E., LASAGNI, L. & ROMAGNANI, P. 2012. Characterization of renal progenitors committed toward tubular lineage and their regenerative potential in renal tubular injury. *Stem Cells,* 30**,** 1714-25.

ARANA, L., GANGOITI, P., OURO, A., TRUEBA, M. & GOMEZ-MUNOZ, A. 2010. Ceramide and ceramide 1-phosphate in health and disease. *Lipids Health Dis,* 9**,** 15.

ARCHIBALD, A. L., BOLUND, L., CHURCHER, C., FREDHOLM, M., GROENEN, M. A., HARLIZIUS, B., LEE, K. T., MILAN, D., ROGERS, J., ROTHSCHILD, M. F., UENISHI, H., WANG, J. & SCHOOK, L. B. 2010. Pig genome sequence--analysis and publication strategy. *BMC Genomics,* 11**,** 438.

AROOJ, A., LAM, J., WUI, Y. J. & SUPRIYANTO, E. 2011. Comparison of Renal Size among Different Ethnicities. Int. J.L Bio Biomed Engin, 5(4), 221-229.

ARTHUR, J. M., HILL, E. G., ALGE, J. L., LEWIS, E. C., NEELY, B. A., JANECH, M. G., TUMLIN, J. A., CHAWLA, L. S. & SHAW, A. D. 2014. Evaluation of 32 urine biomarkers to predict the progression of acute kidney injury after cardiac surgery. *Kidney Int,* 85**,** 431-8.

ARVANITIS, C. & FELSHER, D. W. 2005. Conditionally MYC: insights from novel transgenic models. *Cancer Lett,* 226**,** 95-9.

ARVANITIS, C. & FELSHER, D. W. 2006. Conditional transgenic models define how MYC initiates and maintains tumorigenesis. *Semin Cancer Biol,* 16**,** 313-7.

ASKENAZI, D. 2011. Evaluation and management of critically ill children with acute kidney injury. *Curr Opin Pediatr,* 23**,** 201-7.

AZAD, A. K., AHMED, T., CHOWDHURY, A. J., RAHIM, M. A., MAHMUD, A. K. & RAHMAN, M. A. 2008. Cytomegalovirus induced hepatitis in an immunocompetent host. *Mymensingh Med J,* 17**,** S104-6.

B.J. WITTEKIND, C. C. C. & SOBIN L. H. (EDS). 2012. *A Commentary on Uniform Use. UICC International Union against cancer.*, Wiley-Blackwell.

BABA, M., FURIHATA, M., HONG, S. B., TESSAROLLO, L., HAINES, D. C., SOUTHON, E., PATEL, V., IGARASHI, P., ALVORD, W. G., LEIGHTY, R., YAO, M., BERNARDO, M., ILEVA, L., CHOYKE, P., WARREN, M. B., ZBAR, B., LINEHAN, W. M. & SCHMIDT, L. S. 2008. Kidney-targeted Birt-Hogg-Dub?? gene inactivation in a mouse model: Erk1/2 and Akt-mTOR activation, cell hyperproliferation, and polycystic kidneys. *Journal of the National Cancer Institute,* 100**,** 140-154.

BABIYCHUK, E. B. & DRAEGER, A. 2000. Annexins in cell membrane dynamics. Ca(2+)-regulated association of lipid microdomains. *J Cell Biol,* 150**,** 1113-24.

BACALLAO, R. & FINE, L. G. 1989. Molecular events in the organization of renal tubular epithelium: from nephrogenesis to regeneration. *Am J Physiol,* 257**,** F913-24.

BAGETTI FILHO, H. J. S., PEREIRA-SAMPAIO, M. A., FAVORITO L. A. & SAMPAIO. F. J. B. 2008. Pig Kidney: Anatomical Relationships Between the Renal Venous Arrangement and the Kidney Collecting System.179, 1627-1630.

BAKRIS, G. L. & RITZ, E. 2009. The message for World Kidney Day 2009: hypertension and kidney disease: a marriage that should be prevented. *Clin J Am Soc Nephrol,* 4**,** 517-9.

BALAKUMAR, P., ARORA, M. K., GANTI, S. S., REDDY, J. & SINGH, M. 2009. Recent advances in pharmacotherapy for diabetic nephropathy: current perspectives and future directions. *Pharmacol Res,* 60**,** 24-32.

BAO, H., JIANG, M., ZHU, M., SHENG, F., RUAN, J. & RUAN, C. 2009. Overexpression of Annexin II affects the proliferation, apoptosis, invasion and production of proangiogenic factors in multiple myeloma. *Int J Hematol,* 90**,** 177-85.

BASILE, D. P. 2004. Rarefaction of peritubular capillaries following ischemic acute renal failure: a potential factor predisposing to progressive nephropathy. *Curr Opin Nephrol Hypertens,* 13**,** 1-7.

BASILE, D. P., DONOHOE, D., ROETHE, K. & OSBORN, J. L. 2001. Renal ischemic injury results in permanent damage to peritubular capillaries and influences long-term function. *Am J Physiol Renal Physiol,* 281**,** F887-99.

BASU, R. K., DEVARAJAN, P., WONG, H. & WHEELER, D. S. 2011. An update and review of acute kidney injury in pediatrics. *Pediatr Crit Care Med,* 12**,** 339-47.

BEALL, H. D., MURPHY, A. M., SIEGEL, D., HARGREAVES, R. H., BUTLER, J. & ROSS, D. 1995. Nicotinamide adenine dinucleotide (phosphate): quinone oxidoreductase (DT-diaphorase) as a target for bioreductive antitumor quinones: quinone cytotoxicity and selectivity in human lung and breast cancer cell lines. *Mol Pharmacol,* 48**,** 499-504.

BEDI, D. S., RIELLA, L. V., TULLIUS, S. G. & CHANDRAKER, A. 2010. Animal models of chronic allograft injury: contributions and limitations to understanding the mechanism of long-term graft dysfunction. *Transplantation,* 90**,** 935-44.

BEISLAND, C., HJELLE, K. M., REISAETER, L. A. & BOSTAD, L. 2009. Observation should be considered as an alternative in management of renal masses in older and comorbid patients. *Eur Urol,* 55**,** 1419-27.

BELLOMO, R., KELLUM, J. A. & RONCO, C. 2012. Acute kidney injury. *Lancet,* 380**,** 756-66.

BELLOMO, R., RONCO, C., KELLUM, J. A., MEHTA, R. L. & PALEVSKY, P. 2004. Acute renal failure - definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. *Crit Care,* 8**,** R204-12.

BENNETT, M., DENT, C. L., MA, Q., DASTRALA, S., GRENIER, F., WORKMAN, R., SYED, H., ALI, S., BARASCH, J. & DEVARAJAN, P.

2008. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. *Clin J Am Soc Nephrol,* 3**,** 665-73.

BERNARD-KARGAR, C. & KTORZA, A. 2001. Endocrine pancreas plasticity under physiological and pathological conditions. *Diabetes,* 50 Suppl 1**,** S30-5.

BERNARD, A. M., VYSKOCIL, A. A., MAHIEU, P. & LAUWERYS, R. R. 1987. Assessment of urinary retinol-binding protein as an index of proximal tubular injury. *Clin Chem,* 33**,** 775-9.

BEROUKHIM, R., BRUNET, J. P., DI NAPOLI, A., MERTZ, K. D., SEELEY, A., PIRES, M. M., LINHART, D., WORRELL, R. A., MOCH, H., RUBIN, M. A., SELLERS, W. R., MEYERSON, M., LINEHAN, W. M., KAELIN, W. G., JR. & SIGNORETTI, S. 2009. Patterns of gene expression and copy-number alterations in von-hippel lindau disease-associated and sporadic clear cell carcinoma of the kidney. *Cancer Res,* 69**,** 4674-81.

BERTONCELLO, I. & WILLIAMS, B. 2004. Hematopoietic stem cell characterization by Hoechst 33342 and rhodamine 123 staining. *Methods Mol Biol,* 263**,** 181-200.

BERTOUT, J. A., PATEL, S. A., FRYER, B. H., DURHAM, A. C., COVELLO, K. L., OLIVE, K. P., GOLDSCHMIDT, M. H. & SIMON, M. C. 2009. Heterozygosity for hypoxia inducible factor 1alpha decreases the incidence of thymic lymphomas in a p53 mutant mouse model. *Cancer Res,* 69**,** 3213-20.

BIDANI, A. K. & GRIFFIN, K. A. 2002. Long-term renal consequences of hypertension for normal and diseased kidneys. *Curr Opin Nephrol Hypertens,* 11**,** 73-80.

BINDRA, R. S. & GLAZER, P. M. 2005. Genetic instability and the tumor microenvironment: towards the concept of microenvironment-induced mutagenesis. *Mutat Res,* 569**,** 75-85.

BIRNBAUM, B. A., BOSNIAK, M. A., MEGIBOW, A. J., LUBAT, E. & GORDON, R. B. 1990. Observations on the growth of renal neoplasms. *Radiology,* 176**,** 695-701.

BLANK, M. & SHOENFELD, Y. 2008. Histidine-rich glycoprotein modulation of immune/autoimmune, vascular, and coagulation systems. *Clin Rev Allergy Immunol,* 34**,** 307-12.

BONVENTRE, J. V. 2003. Dedifferentiation and proliferation of surviving epithelial cells in acute renal failure. *J Am Soc Nephrol,* 14 Suppl 1**,** S55-61.

BONVENTRE, J. V. & YANG, L. 2011. Cellular pathophysiology of ischemic acute kidney injury. J Clin Invest, 121: 4210–4221.

BONVENTRE, J. V. & ZUK, A. 2004. Ischemic acute renal failure: an inflammatory disease? *Kidney Int,* 66**,** 480-5.

BORZA, D. B. & MORGAN, W. T. 1997. Acceleration of plasminogen activation by tissue plasminogen activator on surface-bound histidine-proline-rich glycoprotein. *J Biol Chem,* 272**,** 5718-26.

BOSNIAK, M. A., BIRNBAUM, B. A., KRINSKY, G. A. & WAISMAN, J. 1995. Small renal parenchymal neoplasms: further observations on growth. *Radiology,* 197**,** 589-97.

BOUCHARD, M., SOUABNI, A., MANDLER, M., NEUBÜSER, A. & BUSSLINGER, M. 2002. Nephric lineage specification by Pax2 and Pax8. *Genes and Development,* 16**,** 2958-2970.

BOUZAS-MOSQUERA A, V.-R. J. M. P. J. A.-G. N. 2009. Acute kidney injury and long-term prognosis after acute myocardial infarction. *Arch Intern Med.,* 169**,** 87-87.

BOX, A. H. & DEMETRICK, D. J. 2004. Cell cycle kinase inhibitor expression and hypoxia-induced cell cycle arrest in human cancer cell lines. *Carcinogenesis,* 25**,** 2325-35.

BOYER, L. A.. LEE, T. I., COLE, M. F., JOHNSTONE, S. E., LEVINE, S. S., ZUCKER, J. P., GUENTHER, M. G., KUMAR, R. M., MURRAY, H. L., JENNER, R. G., GIFFORD, D. K., MELTON, D. A., JAENISCH, R. & YOUNG, R. A. 2005. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell, 122, 947-956.

BRADEN, A. R., KAFKA, M. T., CUNNINGHAM, L., JONES, H. & VISHWANATHA, J. K. 2009. Polymeric nanoparticles for sustained down-regulation of annexin A2 inhibit prostate tumor growth. *J Nanosci Nanotechnol,* 9**,** 2856-65.

BRETHEAU, D., LECHEVALLIER, E., DE FROMONT, M., SAULT, M. C., RAMPAL, M. & COULANGE, C. 1995. Prognostic value of nuclear grade of renal cell carcinoma. *Cancer,* 76**,** 2543-2549.

BRISTOW, R. G. & HILL, R. P. 2008. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. *Nat Rev Cancer,* 8**,** 180-92.

BRIZEL, D. M., SCULLY, S. P., HARRELSON, J. M., LAYFIELD, L. J., BEAN, J. M., PROSNITZ, L. R. & DEWHIRST, M. W. 1996. Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma. *Cancer Res,* 56**,** 941-3.

BROWN D. & WAGNER, C. A. 2012. Molecular Mechanisms of Acid-Base Sensing by the Kidney. *J Am Soc Nephrol*, 23, 774–780.

BROWN, K. J. & PARISH, C. R. 1994. Histidine-rich glycoprotein and platelet factor 4 mask heparan sulfate proteoglycans recognized by acidic and basic fibroblast growth factor. *Biochemistry,* 33**,** 13918-27.

BRUGAROLAS, J. 2014. Molecular genetics of clear-cell renal cell carcinoma. *J Clin Oncol,* 32**,** 1968-76.

BRUNO, S., BUSSOLATI, B., GRANGE, C., COLLINO, F., GRAZIANO, M. E., FERRANDO, U. & CAMUSSI, G. 2006. CD133+ renal progenitor cells contribute to tumor angiogenesis. *Am J Pathol,* 169**,** 2223-35.

BUCALOIU, I. D., KIRCHNER, H. L., NORFOLK, E. R., HARTLE, J. E. & PERKINS, R. M. 2012. Increased risk of death and de novo chronic kidney disease following reversible acute kidney injury. *Kidney Int,* 81**,** 477-485.

BUI, M. H., SELIGSON, D., HAN, K. R., PANTUCK, A. J., DOREY, F. J., HUANG, Y., HORVATH, S., LEIBOVICH, B. C., CHOPRA, S., LIAO, S. Y., STANBRIDGE, E., LERMAN, M. I., PALOTIE, A., FIGLIN, R. A. & BELLDEGRUN, A. S. 2003. Carbonic anhydrase IX is an independent predictor of survival in advanced renal clear cell carcinoma: implications for prognosis and therapy. *Clin Cancer Res,* 9**,** 802-11.

BUSSOLATI, B., BRUNO, S., GRANGE, C., BUTTIGLIERI, S., DEREGIBUS, M. C., CANTINO, D. & CAMUSSI, G. 2005. Isolation of renal progenitor cells from adult human kidney. *Am J Pathol,* 166**,** 545-55.

BUSSOLATI, B., DEKEL, B., AZZARONE, B. & CAMUSSI, G. 2013. Human renal cancer stem cells. *Cancer Lett,* 338**,** 141-6.

BUSSOLATI, B., MOGGIO, A., COLLINO, F., AGHEMO, G., D'ARMENTO, G., GRANGE, C. & CAMUSSI, G. 2012. Hypoxia modulates the undifferentiated phenotype of human renal inner medullary CD133+ progenitors through Oct4/miR-145 balance. *Am J Physiol Renal Physiol,* 302**,** F116-28.

CALLONI, R., CORDERO, E. A. A., HENRIQUES, J. A. P & BONATTO, D. 2013. Updating the Major Molecular Markers for Stem Cells. Stem Cells Dev, 22, 1455-1475.

CARAMORI, M. L. & MAUER, M. 2003. Diabetes and nephropathy. *Curr Opin Nephrol Hypertens,* 12**,** 273-82.

CARLSON, B. M. 2004. *Human Embryology and Developmental Biology*, Elsevier Science.

CARMELIET, P., DOR, Y., HERBERT, J. M., FUKUMURA, D., BRUSSELMANS, K., DEWERCHIN, M., NEEMAN, M., BONO, F., ABRAMOVITCH, R., MAXWELL, P., KOCH, C. J., RATCLIFFE, P., MOONS, L., JAIN, R. K., COLLEN, D. & KESHERT, E. 1998. Role of HIF-1alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. *Nature,* 394**,** 485-90.

CARRAFIELLO, G., LAGANA, D., IANNIELLO, A., MANGINI, M., FONTANA, F., COTTA, E., CONCOLLATO, L., MARCONI, A., RECALDINI, C., DIONIGI, G., ROVERA, F., BONI, L., CUFFARI, S. & FUGAZZOLA, C. 2008. Percutaneous radiofrequency thermal ablation of renal cell carcinoma: is it possible a day-hospital treatment? *Int J Surg,* 6 Suppl 1**,** S31-5.

CARROLL, J. S., SWARBRICK, A., MUSGROVE, E. A. & SUTHERLAND, R. L. 2002. Mechanisms of growth arrest by c-myc antisense oligonucleotides in MCF-7 breast cancer cells: implications for the antiproliferative effects of antiestrogens. *Cancer Res,* 62**,** 3126-31.

CERAMI, E., GAO, J., DOGRUSOZ, U., GROSS, B. E., SUMER, S. O., AKSOY, B. A., JACOBSEN, A., BYRNE, C. J., HEUER, M. L., LARSSON, E., ANTIPIN, Y., REVA, B., GOLDBERG, A. P., SANDER, C. & SCHULTZ, N. 2012. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov,* 2**,** 401-4.

CERDÁ, J., BAGGA, A., KHER, V. & CHAKRAVARTHI, R. M. 2008. The contrasting characteristics of acute kidney injury in developed and developing countries. *Nature clinical practice. Nephrology,* 4**,** 138-53.

CERSOSIMO, E., SOLIS-HERRERA, C. & TRIPLITT, C. 2014. Inhibition of renal glucose reabsorption as a novel treatment for diabetes patients. *J Bras Nefrol*, 36(1), 80-92.

CHALLEN, G. A., BERTONCELLO, I., DEANE, J. A., RICARDO, S. D. & LITTLE, M. H. 2006. Kidney side population reveals multilineage potential and renal functional capacity but also cellular heterogeneity. *J Am Soc Nephrol,* 17**,** 1896-912.

CHAN, D. A. & GIACCIA, A. J. 2007. Hypoxia, gene expression, and metastasis. *Cancer Metastasis Rev,* 26**,** 333-9.

CHANG, N. S., LEU, R. W., ANDERSON, J. K. & MOLE, J. E. 1994. Role of N-terminal domain of histidine-rich glycoprotein in modulation of macrophage Fc gamma receptor-mediated phagocytosis. *Immunology,* 81**,** 296-302.

CHANG, N. S., LEU, R. W., RUMMAGE, J. A., ANDERSON, J. K. & MOLE, J. E. 1992. Regulation of macrophage Fc receptor expression and phagocytosis by histidine-rich glycoprotein. *Immunology,* 77**,** 532-8.

CHAWLA, L. S., AMDUR, R. L., AMODEO, S., KIMMEL, P. L. & PALANT, C. E. 2011. The severity of acute kidney injury predicts progression to chronic kidney disease. *Kidney international,* 79**,** 1361-9.

CHEADLE, J. P., REEVE, M. P., SAMPSON, J. R. & KWIATKOWSKI, D. J. 2000. Molecular genetic advances in tuberous sclerosis. *Hum Genet,* 107**,** 97-114.

CHEN, J., ROCKEN, C., HOFFMANN, J., KRUGER, S., LENDECKEL, U., ROCCO, A., PASTOREKOVA, S., MALFERTHEINER, P. & EBERT, M. P. 2005. Expression of carbonic anhydrase 9 at the invasion front of gastric cancers. *Gut,* 54**,** 920-7.

CHERTOW, G. M., BURDICK, E., HONOUR, M., BONVENTRE, J. V. & BATES, D. W. 2005. Acute kidney injury, mortality, length of stay, and costs in hospitalized patients. *J Am Soc Nephrol,* 16**,** 3365-3370.

CHERTOW, G. M., CHRISTIANSEN, C. L., CLEARY, P. D., MUNRO, C. & LAZARUS, J. M. 1995. Prognostic stratification in critically ill patients with acute renal failure requiring dialysis. *Arch Intern Med,* 155**,** 1505-11.

CHEVILLE, J. C., LOHSE, C. M., ZINCKE, H., WEAVER, A. L. & BLUTE, M. L. 2003. Comparisons of outcome and prognostic features among histologic subtypes of renal cell carcinoma. *The American journal of surgical pathology,* 27**,** 612-624.

CHIANG, Y., RIZZINO, A., SIBENALLER, Z. A., WOLD, M. S. & VISHWANATHA, J. K. 1999. Specific down-regulation of annexin II expression in human cells interferes with cell proliferation. *Mol Cell Biochem,* 199**,** 139-47.

CHOI, K. S., FITZPATRICK, S. L., FILIPENKO, N. R., FOGG, D. K., KASSAM, G., MAGLIOCCO, A. M. & WAISMAN, D. M. 2001. Regulation of plasmin-dependent fibrin clot lysis by annexin II heterotetramer. *J Biol Chem,* 276**,** 25212-21.

CHOI, S., PARK, M., KIM, J., HWANG, S., PARK, S. & LEE, Y. 2009. The role of mesenchymal stem cells in the functional improvement of chronic renal failure. *Stem Cells Dev,* 18**,** 521-9.

CHOYKE, P. L. 2003. Imaging of hereditary renal cancer. *Radiol Clin North Am,* 41**,** 1037-51.

CHUNG, K. T. & GADUPUDI, G. S. 2011. Possible roles of excess tryptophan metabolites in cancer.

COCA, S. G., SINGANAMALA, S. & PARIKH, C. R. 2012. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. *Kidney Int,* 81**,** 442-8.

COCA, S. G., YUSUF, B., SHLIPAK, M. G., GARG, A. X. & PARIKH, C. R. 2009. Long-term risk of mortality and other adverse outcomes after acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis,* 53**,** 961-73.

COPPIN, C., KOLLMANNSBERGER, C., LE, L., PORZSOLT, F. & WILT, T. J. 2011. Targeted therapy for advanced renal cell cancer (RCC): a Cochrane systematic review of published randomised trials. *BJU Int,* 108**,** 1556-63.

CORESH, J., SELVIN, E., STEVENS, L. A., MANZI, J., KUSEK, J. W., EGGERS, P., VAN LENTE, F. & LEVEY, A. S. 2007. Prevalence of chronic kidney disease in the United States. *JAMA,* 298**,** 2038-47.

COSTANTINI, F. & KOPAN, R. 2010. Patterning a complex organ: Branching morphogenesis and nephron segmentation in kidney development.

CREIGHTON CJ, M. M., GUNARATNE PH, WHEELER DA, GIBBS RA, ROBERTSON A, CHU A, BEROUKHIM R, CIBULSKIS K, SIGNORETTI S, VANDIN F, WU HT, RAPHAEL BJ, VERHAAK RG, TAMBOLI P, TORRES-GARCIA W, AKBANI R, WEINSTEIN JN, REUTER V, HSIEH JJ, BRANNON A, HAKIMI A, ET AL 2013. Comprehensive molecular characterization of clear cell renal cell carcinoma. *Nature,* 499**,** 43-9.

CRESTEIL, T. & JAISWAL, A. K. 1991. High levels of expression of the NAD(P)H:quinone oxidoreductase (NQO1) gene in tumor cells compared to normal cells of the same origin. *Biochem Pharmacol,* 42**,** 1021-7.

CROTTY, T. B., FARROW, G. M. & LIEBER, M. M. 1995. Chromophobe cell renal carcinoma: clinicopathological features of 50 cases. *The Journal of urology,* 154**,** 964-967.

CRUZ, D. N., BAGSHAW, S. M., RONCO, C. & RICCI, Z. 2010. Acute kidney injury: Classification and staging. *Contributions to Nephrology,* 164**,** 24-32.

DANG, C. V. 1999. c-Myc target genes involved in cell growth, apoptosis, and metabolism. *Mol Cell Biol,* 19**,** 1-11.

DANG, C. V., O'DONNELL, K. A., ZELLER, K. I., NGUYEN, T., OSTHUS, R. C. & LI, F. 2006. The c-Myc target gene network. *Semin Cancer Biol,* 16**,** 253-64.

DAVIS, C. F., RICKETTS, C. J., WANG, M., YANG, L., CHERNIACK, A. D., SHEN, H., BUHAY, C., KANG, H., KIM, S., FAHEY, C. C., HACKER, K. E., BHANOT, G., GORDENIN, D. A., CHU, A., GUNARATNE, P. H., BIEHL, M., SETH, S., KAIPPARETTU, B. A., BRISTOW, C. A., DONEHOWER, L. A., WALLEN, E. M., SMITH, A. B., TICKOO, S. K., TAMBOLI, P., REUTER, V., SCHMIDT, L. S., HSIEH, J. J., CHOUEIRI, T. K., HAKIMI, A. A., CHIN, L., MEYERSON, M., KUCHERLAPATI, R., PARK, W. Y., ROBERTSON, A. G., LAIRD, P. W., HENSKE, E. P., KWIATKOWSKI, D. J., PARK, P. J., MORGAN, M., SHUCH, B., MUZNY, D., WHEELER, D. A., LINEHAN, W. M., GIBBS, R. A., RATHMELL, W. K. & CREIGHTON, C. J. 2014. The Somatic Genomic Landscape of Chromophobe Renal Cell Carcinoma. *Cancer Cell,* 26**,** 319-330.

DE MENDONÇA, A., VINCENT, J. L., SUTER, P. M., MORENO, R., DEARDEN, N. M., ANTONELLI, M., TAKALA, J., SPRUNG, C. & CANTRAINE, F. 2000. Acute renal failure in the ICU: risk factors and outcome evaluated by the SOFA score. *Intensive care medicine,* 26**,** 915-921.

DECLERCK, Y. A., MERCURIO, A. M., STACK, M. S., CHAPMAN, H. A., ZUTTER, M. M., MUSCHEL, R. J., RAZ, A., MATRISIAN, L. M., SLOANE, B. F., NOEL, A., HENDRIX, M. J., COUSSENS, L. & PADARATHSINGH, M. 2004. Proteases, extracellular matrix, and cancer: a workshop of the path B study section. *Am J Pathol,* 164**,** 1131-9.

DEFRONZO, R. A., DAVIDSON, J. A. & PRATO, S. D. 2012.The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. Diabetes, Obesity and Metabol, 14: 5–14.

DEHOUX, J. P. & GIANELLO, P. 2007. The importance of large animal models in transplantation. *Front Biosci,* 12**,** 4864-80.

DELAHUNT, B. & EBLE, J. N. 1997. Papillary renal cell carcinoma: a clinicopathologic and immunohistochemical study of 105 tumors. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc,* 10**,** 537-44.

DENT, C. L., MA, Q., DASTRALA, S., BENNETT, M., MITSNEFES, M. M., BARASCH, J. & DEVARAJAN, P. 2007. Plasma neutrophil gelatinase-associated lipocalin predicts acute kidney injury, morbidity and mortality after pediatric cardiac surgery: a prospective uncontrolled cohort study. *Crit Care,* 11**,** R127.

DEVARAJAN, P. 2006. Update on Mechanisms of Ischemic Acute Kidney Injury. J Am Soc Nephrol, 17, 1503–1520.

DEVARAJAN, P. 2008. The future of pediatric acute kidney injury management--biomarkers. *Semin Nephrol,* 28**,** 493-8.

DEVARAJAN, P. 2011. Biomarkers for the early detection of acute kidney injury. *Curr Opin Pediatr,* 23**,** 194-200.

DI NEZZA, L. A., MISAJON, A., ZHANG, J., JOBLING, T., QUINN, M. A., OSTOR, A. G., NIE, G., LOPATA, A. & SALAMONSEN, L. A. 2002. Presence of active gelatinases in endometrial carcinoma and correlation of matrix metalloproteinase expression with increasing tumor grade and invasion. *Cancer,* 94**,** 1466-75.

DISCENZA, M. T., HE, S., LEE, T. H., CHU, L. L.,, BOLON, B., GOODYER, P., ECCLES M. & PELLETIER, J. 2003. WT1 is a modifier of the Pax2 mutant phenotype: cooperation and interaction between WT1 and Pax2. Oncogene, 22, 8145–8155.

DONATE, F., JUAREZ, J. C., GUAN, X., SHIPULINA, N. V., PLUNKETT, M. L., TEL-TSUR, Z., SHAW, D. E., MORGAN, W. T. & MAZAR, A. P. 2004. Peptides derived from the histidine-proline domain of the histidine-proline-rich glycoprotein bind to tropomyosin and have antiangiogenic and antitumor activities. *Cancer Res,* 64**,** 5812-7.

DOSLUOGLU, H. H., AKTAN, A. O., YEGEN, C., OKBOY, N., YALCM, A. S., YAHN, R. & ERCAN, S. 1993. The cytoprotective effects of verapamil and iloprost (ZK 36374) on ischemia/reperfusion injury of kidneys. *Transpl Int,* 6**,** 138-42.

DRABKIN, H. A., BRADLEY, C., HART, I., BLESKAN, J., LI, F. P. & PATTERSON, D. 1985. Translocation of c-myc in the hereditary renal cell carcinoma associated with a t(3;8)(p14.2;q24.13) chromosomal translocation. *Proc Natl Acad Sci U S A,* 82**,** 6980-4.

DRESSLER, G. R. 2006. The cellular basis of kidney development. *Annu Rev Cell Dev Biol,* 22**,** 509-29.

DU, Y., ZAPPITELLI, M., MIAN, A., BENNETT, M., MA, Q., DEVARAJAN, P., MEHTA, R. & GOLDSTEIN, S. L. 2011. Urinary biomarkers to detect acute kidney injury in the pediatric emergency center. *Pediatr Nephrol,* 26**,** 267-74.

DUFFIELD, J. S., PARK, K. M., HSIAO, L. L., KELLEY, V. R., SCADDEN, D. T., ICHIMURA, T. & BONVENTRE, J. V. 2005. Restoration of tubular epithelial cells during repair of the postischemic kidney occurs independently of bone marrow-derived stem cells. *J Clin Invest,* 115**,** 1743-55.

DUQUE, J. L., LOUGHLIN, K. R., O'LEARY, M. P., KUMAR, S. & RICHIE, J. P. 1998. Partial nephrectomy: alternative treatment for selected patients with renal cell carcinoma. *Urology,* 52**,** 584-590.

DURAND, R. E. 1991. Keynote address: the influence of microenvironmental factors on the activity of radiation and drugs. *Int J Radiat Oncol Biol Phys,* 20**,** 253-8.

DUZOVA, A., BAKKALOGLU, A., KALYONCU, M., POYRAZOGLU, H., DELIBAS, A., OZKAYA, O., PERU, H., ALPAY, H., SOYLEMEZOGLU, O., GUR-GUVEN, A., BAK, M., BIRCAN, Z., CENGIZ, N., AKIL, I., OZCAKAR, B., UNCU, N., KARABAY-BAYAZIT, A. & SONMEZ, F. 2010. Etiology and outcome of acute kidney injury in children. *Pediatr Nephrol,* 25**,** 1453-61.

EAVES, H. L. & GAO, Y. 2009. MOM: maximum oligonucleotide mapping. *Bioinformatics,* 25**,** 969-70.

EBRHARD, D. A., KARNS, L. R., VANDENBERG, S. R. & CREUTZ, C. E. 2001. Control of the nuclear-cytoplasmic partitioning of annexin II by a nuclear export signal and by p11 binding. *J Cell Sci,* 114**,** 3155-66.

EDDY, A. A. & NEILSON, E. G. 2006. Chronic kidney disease progression. *J Am Soc Nephrol,* 17**,** 2964-6.

EGEBLAD, M. & WERB, Z. 2002. New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer,* 2**,** 161-74.

EL-SHARKAWYA, A. M., SAHOTA, O., MAUGHAN, R. J. & LOBO, D. N. 2014. The pathophysiology of fluid and electrolyte balance in the older adult surgical patient. Clinical Nutrition, 33, 6e-13

EMANS, N., GORVEL, J. P., WALTER, C., GERKE, V., KELLNER, R., GRIFFITHS, G. & GRUENBERG, J. 1993. Annexin II is a major component of fusogenic endosomal vesicles. *J Cell Biol,* 120**,** 1357-69.

EMOTO, K., SAWADA, H., YAMADA, Y., FUJIMOTO, H., TAKAHAMA, Y., UENO, M., TAKAYAMA, T., UCHIDA, H., KAMADA, K., NAITO, A., HIRAO, S. & NAKAJIMA, Y. 2001a. Annexin II overexpression is correlated with poor prognosis in human gastric carcinoma. *Anticancer Res,* 21**,** 1339-45.

EMOTO, K., YAMADA, Y., SAWADA, H., FUJIMOTO, H., UENO, M., TAKAYAMA, T., KAMADA, K., NAITO, A., HIRAO, S. & NAKAJIMA, Y. 2001b. Annexin II overexpression correlates with stromal tenascin-C overexpression: a prognostic marker in colorectal carcinoma. *Cancer,* 92**,** 1419-26.

ERKASAP, S., ERKASAP, N., KOKEN, T., KAHRAMAN, A., UZUNER, K., YAZIHAN, N. & ATES, E. 2004. Effect of leptin on renal ischemia-reperfusion damage in rats. *J Physiol Biochem,* 60**,** 79-84.

ESKICORAPCI, S. Y., TEBER, D., SCHULZE, M., ATES, M., STOCK, C. & RASSWEILER, J. J. 2007. Laparoscopic radical nephrectomy: The new gold standard surgical treatment for localized renal cell carcinoma. *Thescientificworldjournal,* 7**,** 825-836.

ESPOSITO, I., PENZEL, R., CHAIB-HARRIRECHE, M., BARCENA, U., BERGMANN, F., RIEDL, S., KAYED, H., GIESE, N., KLEEFF, J., FRIESS, H. & SCHIRMACHER, P. 2006. Tenascin C and annexin II expression in the process of pancreatic carcinogenesis. *J Pathol,* 208**,** 673-85.

FAN, X., KRAHLING, S., SMITH, D., WILLIAMSON, P. & SCHLEGEL, R. A. 2004. Macrophage surface expression of annexins I and II in the phagocytosis of apoptotic lymphocytes. *Mol Biol Cell,* 15**,** 2863-72.

FAN, Y., LIU, Z., FANG, X., GE, Z., GE, N., JIA, Y., SUN, P., LOU, F., BJORKHOLM, M., GRUBER, A., EKMAN, P. & XU, D. 2005. Differential expression of full-length telomerase reverse transcriptase mRNA and telomerase activity between normal and malignant renal tissues. *Clin Cancer Res,* 11**,** 4331-7.

FELSHER, D. W. & BISHOP, J. M. 1999a. Reversible tumorigenesis by MYC in hematopoietic lineages. *Mol Cell,* 4**,** 199-207.

FELSHER, D. W. & BISHOP, J. M. 1999b. Transient excess of MYC activity can elicit genomic instability and tumorigenesis. *Proc Natl Acad Sci U S A,* 96**,** 3940-4.

FICARRA, V., MARTIGNONI, G., MAFFEI, N., BRUNELLI, M., NOVARA, G., ZANOLLA, L., PEA, M. & ARTIBANI, W. 2005. Original and reviewed nuclear grading according to the Fuhrman system: A multivariate analysis of 388 patients with conventional renal cell carcinoma.

FINN, W. F. 1993. *Finn WF.1993 Recovery from acute renal failure. In: Acute Renal Failure* New York.

FIORETTO, P., CARAMORI, M. L. & MAUER, M. 2008. The kidney in diabetes: dynamic pathways of injury and repair. The Camillo Golgi Lecture 2007. *Diabetologia,* 51**,** 1347-55.

FLOCKS RH, K. M. C. 1958. Malignant neoplasms of the kidney; an analysis of 353 patients followed five years or more. *J Urol,* 79**,** 196-201.

FORBES, S. J., POULSOM, R. & WRIGHT, N. A. 2002. Hepatic and renal differentiation from blood-borne stem cells. *Gene Ther,* 9**,** 625-30.

FOSSÅ, S. D., KJØLSETH, I. & LUND, G. 1982. Radiotherapy of metastases from renal cancer. *European urology,* 8**,** 340-2.

FRITZBERG, A. R. 1984. Current Status of Renal Radiopharmaceuticals. J Nucl Med Techol, 12 (4), 177-188.

FU, L., MINTON, D. R., ZHANG, T., NANUS, D. M. & GUDAS, L. J. 2015. Genome-Wide Profiling of TRACK Kidneys Shows Similarity to the Human ccRCC Transcriptome. *Mol Cancer Res,* 13**,** 870-8.

FU, L., WANG, G., SHEVCHUK, M. M., NANUS, D. M. & GUDAS, L. J. 2011. Generation of a mouse model of Von Hippel-Lindau kidney disease leading to renal cancers by expression of a constitutively active mutant of HIF1alpha. *Cancer Res,* 71**,** 6848-56.

FU, L., WANG, G., SHEVCHUK, M. M., NANUS, D. M. & GUDAS, L. J. 2013. Activation of HIF2alpha in kidney proximal tubule cells causes abnormal glycogen deposition but not tumorigenesis. *Cancer Res,* 73**,** 2916-25.

FUHRMAN SA, L. L. C. L. C. 1982. Prognostic significance of morphologic parameters in renal cell carcinoma. *Am J Surg Pathol.,* 6**,** 655-63.

FURGE, K. A., CHEN, J., KOEMAN, J., SWIATEK, P., DYKEMA, K., LUCIN, K., KAHNOSKI, R., YANG, X. J. & TEH, B. T. 2007. Detection of DNA copy number changes and oncogenic signaling abnormalities from gene expression data reveals MYC activation in high-grade papillary renal cell carcinoma. *Cancer Res,* 67**,** 3171-6.

GAO, J., AKSOY, B. A., DOGRUSOZ, U., DRESDNER, G., GROSS, B., SUMER, S. O., SUN, Y., JACOBSEN, A., SINHA, R., LARSSON, E., CERAMI, E., SANDER, C. & SCHULTZ, N. 2013. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal,* 6**,** pl1.

GARDNER, D. S., DE BROT, S., DUNFORD, L. J., GRAU-ROMA, L., WELHAM, S. J., FALLMAN, R., O'SULLIVAN, S. E., OH, W. & DEVONALD, M. A. 2016. Remote effects of acute kidney injury in a porcine model. *Am J Physiol Renal Physiol,* 310**,** F259-71.

GARDNER, D. S., WELHAM, S. J., DUNFORD, L. J., MCCULLOCH, T. A., HODI, Z., SLEEMAN, P., O'SULLIVAN, S. & DEVONALD, M. A. 2014. Remote conditioning or erythropoietin before surgery primes kidneys to clear ischemia-reperfusion-damaged cells: a renoprotective mechanism? *Am J Physiol Renal Physiol,* 306**,** F873-84.

GENG, H., LAN, R., WANG, G., SIDDIQI, A. R., NASKI, M. C., BROOKS, A. I., BARNES, J. L., SAIKUMAR, P., WEINBERG, J. M. & VENKATACHALAM, M. A. 2009. Inhibition of autoregulated TGFbeta signaling simultaneously enhances proliferation and differentiation of kidney epithelium and promotes repair following renal ischemia. *Am J Pathol,* 174**,** 1291-308.

GEZ, E., LIBES, M., BAR-DEROMA, R., RUBINOV, R., STEIN, M. & KUTEN, A. 2002. Postoperative irradiation in localized renal cell carcinoma: The Rambam Medical Center experience. *Tumori,* 88**,** 500-502.

GIACCIA, A. J. 1996. Hypoxic Stress Proteins: Survival of the Fittest. *Semin Radiat Oncol,* 6**,** 46-58.

GILBERT, B. R., LESLIE, B. R., VAUGHAN, E. D. JR., 1992. Cambell's Urology: Normal Renal Physiology. ed. PC WALSH, AB RETIK, TA STAMEY AND E.D. VAUGHAN, JR.W. B. SAUNDERS CO., 6th Edition 1(2), 70-90.

GIRAUD, S., FAVREAU, F., CHATAURET, N., THUILLIER, R., MAIGA, S. & HAUET, T. 2011. Contribution of large pig for renal ischemia-reperfusion and transplantation studies: the preclinical model. *J Biomed Biotechnol,* 2011**,** 532127.

GIURIATO, S., RYEOM, S., FAN, A. C., BACHIREDDY, P., LYNCH, R. C., RIOTH, M. J., VAN RIGGELEN, J., KOPELMAN, A. M., PASSEGUE, E., TANG, F., FOLKMAN, J. & FELSHER, D. W. 2006. Sustained regression of tumors upon MYC inactivation requires p53 or thrombospondin-1 to reverse the angiogenic switch. *Proc Natl Acad Sci U S A,* 103**,** 16266-71.

GNARRA, J. R., TORY, K., WENG, Y., SCHMIDT, L., WEI, M. H., LI, H., LATIF, F., LIU, S., CHEN, F., DUH, F. M. & ET AL. 1994. Mutations of the VHL tumour suppressor gene in renal carcinoma. *Nat Genet,* 7**,** 85-90.

GODA, N., RYAN, H. E., KHADIVI, B., MCNULTY, W., RICKERT, R. C. & JOHNSON, R. S. 2003. Hypoxia-inducible factor 1alpha is essential for cell cycle arrest during hypoxia. *Mol Cell Biol,* 23**,** 359-69.

GOLDSTEIN, S. L. 2011. Continuous renal replacement therapy: mechanism of clearance, fluid removal, indications and outcomes. *Curr Opin Pediatr,* 23**,** 181-5.

GOMES, D., AGASSE, A., THIÉBAUD, P., DELROT, S., GEROS, H. & CHAUMONT, F. 2009.Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. Biochimica et Biophysica Acta, 1788, 1213–1228.

GORDAN, J. D., LAL, P., DONDETI, V. R., LETRERO, R., PAREKH, K. N., OQUENDO, C. E., GREENBERG, R. A., FLAHERTY, K. T., RATHMELL, W. K., KEITH, B., SIMON, M. C. & NATHANSON, K. L. 2008. HIF-alpha effects on c-Myc distinguish two subtypes of sporadic VHL-deficient clear cell renal carcinoma. *Cancer Cell,* 14**,** 435-46.

GORGANI, N. N., ALTIN, J. G. & PARISH, C. R. 1999. Histidine-rich glycoprotein prevents the formation of insoluble immune complexes by rheumatoid factor. *Immunology,* 98**,** 456-63.

GORGANI, N. N., EASTERBROOK-SMITH, S. B. & ALTIN, J. G. 1996. The formation of insoluble immune complexes between ovalbumin and anti-ovalbumin IgG occurs in at least two distinct phases dependent on reactant concentration and ionic strength. *Biochim Biophys Acta,* 1317**,** 45-54.

GORGANI, N. N., PARISH, C. R., EASTERBROOK SMITH, S. B. & ALTIN, J. G. 1997. Histidine-rich glycoprotein binds to human IgG and C1q and inhibits the formation of insoluble immune complexes. *Biochemistry,* 36**,** 6653-62.

GORGANI, N. N., SMITH, B. A., KONO, D. H. & THEOFILOPOULOS, A. N. 2002. Histidine-rich glycoprotein binds to DNA and Fc gamma RI and potentiates the ingestion of apoptotic cells by macrophages. *J Immunol,* 169**,** 4745-51.

GOURISHANKAR, S., COURTNEY, M., JHANGRI, G. S., CEMBROWSKI, G. & PANNU, N. 2008. Serum cystatin C performs similarly to traditional markers of kidney function in the evaluation of donor kidney function prior to and following unilateral nephrectomy. *Nephrol Dial Transplant,* 23**,** 3004-9.

GRABMAIER, K., MC, A. D. W., VERHAEGH, G. W., SCHALKEN, J. A. & OOSTERWIJK, E. 2004. Strict regulation of CAIX(G250/MN) by HIF-1alpha in clear cell renal cell carcinoma. *Oncogene,* 23**,** 5624-31.

GREENE, F., PAGE, D., FLEMING, I. & FRITZ, A. 2002. American Joint Committee on Cancer. *AJCC cancer staging …***,** 387-390.

GRIFFIN, K. A., KRAMER, H. & BIDANI, A. K. 2008. Adverse renal consequences of obesity. *Am J Physiol Renal Physiol,* 294**,** F685-96.

GUAN, X., JUAREZ, J. C., QI, X., SHIPULINA, N. V., SHAW, D. E., MORGAN, W. T., MCCRAE, K. R., MAZAR, A. P. & DONATE, F. 2004. Histidine-proline rich glycoprotein (HPRG) binds and transduces anti-angiogenic signals through cell surface tropomyosin on endothelial cells. *Thromb Haemost,* 92**,** 403-12.

GUDAS, L. J., FU, L., MINTON, D. R., MONGAN, N. P. & NANUS, D. M. 2014. The role of HIF1alpha in renal cell carcinoma tumorigenesis. *J Mol Med (Berl),* 92**,** 825-36.

GUELER, F., GWINNER, W., SCHWARZ, A. & HALLER, H. 2004. Long-term effects of acute ischemia and reperfusion injury. *Kidney Int,* 66**,** 523-7.

GUMZ, M. L., ZOU, H., KREINEST, P. A., CHILDS, A. C., BELMONTE, L. S., LEGRAND, S. N., WU, K. J., LUXON, B. A., SINHA, M., PARKER, A. S., SUN, L. Z., AHLQUIST, D. A., WOOD, C. G. & COPLAND, J. A. 2007. Secreted frizzled-related protein 1 loss contributes to tumor phenotype of clear cell renal cell carcinoma. *Clin Cancer Res,* 13**,** 4740-9.

GUPTA, S., VERFAILLIE, C., CHMIELEWSKI, D., KIM, Y. & ROSENBERG, M. E. 2002. A role for extrarenal cells in the regeneration following acute renal failure. *Kidney Int,* 62**,** 1285-90.

GUYTON, A.C. 1996. Textbook of Medical Physiology. 9'th edition, W.B. SAUNDERS & CO., USA, 937-951.

HAASE, M., BELLOMO, R., DEVARAJAN, P., SCHLATTMANN, P. & HAASE-FIELITZ, A. 2009. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis,* 54**,** 1012-24.

HAASE, M., DEVARAJAN, P., HAASE-FIELITZ, A., BELLOMO, R., CRUZ, D. N., WAGENER, G., KRAWCZESKI, C. D., KOYNER, J. L., MURRAY, P., ZAPPITELLI, M., GOLDSTEIN, S. L., MAKRIS, K., RONCO, C., MARTENSSON, J., MARTLING, C. R., VENGE, P., SIEW, E., WARE, L. B., IKIZLER, T. A. & MERTENS, P. R. 2011. The outcome of neutrophil gelatinase-associated lipocalin-positive subclinical acute kidney injury: a multicenter pooled analysis of prospective studies. *J Am Coll Cardiol,* 57**,** 1752-61.

HAJJAR, D. P., BOYD, D. B., HARPEL, P. C. & NACHMAN, R. L. 1987. Histidine-rich glycoprotein inhibits the antiproliferative effect of heparin on smooth muscle cells. *J Exp Med,* 165**,** 908-13.

HAJJAR, K. A. & KRISHNAN, S. 1999. Annexin II: a mediator of the plasmin/plasminogen activator system. *Trends Cardiovasc Med,* 9**,** 128-38.

HAJJAR, K. A., MAURI, L., JACOVINA, A. T., ZHONG, F., MIRZA, U. A., PADOVAN, J. C. & CHAIT, B. T. 1998. Tissue plasminogen activator

binding to the annexin II tail domain. Direct modulation by homocysteine. *J Biol Chem,* 273**,** 9987-93.

HALL, I. E., COCA, S. G., PERAZELLA, M. A., EKO, U. U., LUCIANO, R. L., PETER, P. R., HAN, W. K. & PARIKH, C. R. 2011. Risk of poor outcomes with novel and traditional biomarkers at clinical AKI diagnosis. *Clin J Am Soc Nephrol,* 6**,** 2740-9.

HANAHAN, D. & WEINBERG, R. A. 2000. The hallmarks of cancer. *Cell,* 100**,** 57-70.

HANAHAN, D. & WEINBERG, R. A. 2011a. Hallmarks of cancer: The next generation.

HANAHAN, D. & WEINBERG, R. A. 2011b. Hallmarks of cancer: the next generation. *Cell,* 144**,** 646-74.

HANSSON, J., ERICSSON, A. E., AXELSON, H. & JOHANSSON, M. E. 2016. Species diversity regarding the presence of proximal tubular progenitor cells of the kidney. *Eur J Histochem,* 60**,** 2567.

HANSSON, J., HULTENBY, K., CRAMNERT, C., PONTEN, F., JANSSON, H., LINDGREN, D., AXELSON, H. & JOHANSSON, M. E. 2014. Evidence for a morphologically distinct and functionally robust cell type in the proximal tubules of human kidney. *Hum Pathol,* 45**,** 382-93.

HARA, Y., STOLK, M., RINGE, J., DEHNE, T., LADHOFF, J., KOTSCH, K., REUTZEL-SELKE, A., REINKE, P., VOLK, H. D. & SEIFERT, M. 2011. In vivo effect of bone marrow-derived mesenchymal stem cells in a rat kidney transplantation model with prolonged cold ischemia. *Transpl Int,* 24**,** 1112-23.

HAROON, Z. A., RALEIGH, J. A., GREENBERG, C. S. & DEWHIRST, M. W. 2000. Early wound healing exhibits cytokine surge without evidence of hypoxia. *Ann Surg,* 231**,** 137-47.

HARRIS, A. L. 2002. Hypoxia--a key regulatory factor in tumour growth. *Nat Rev Cancer,* 2**,** 38-47.

HE, T. C., SPARKS, A. B., RAGO, C., HERMEKING, H., ZAWEL, L., DA COSTA, L. T., MORIN, P. J., VOGELSTEIN, B. & KINZLER, K. W. 1998. Identification of c-MYC as a target of the APC pathway. *Science,* 281**,** 1509-12.

HERMAN, J. G., LATIF, F., WENG, Y., LERMAN, M. I., ZBAR, B., LIU, S., SAMID, D., DUAN, D. S., GNARRA, J. R., LINEHAN, W. M. & ET AL. 1994. Silencing of the VHL tumor-suppressor gene by DNA methylation in renal carcinoma. *Proc Natl Acad Sci U S A,* 91**,** 9700-4.

HERNANDEZ-YANEZ, M., HEYMACH, J. V. & ZURITA, A. J. 2012. Circulating biomarkers in advanced renal cell carcinoma: clinical applications. *Curr Oncol Rep,* 14**,** 221-9.

HERZLINGER, D., KOSEKI, C., MIKAWA, T. & AL-AWQATI, Q. 1992. Metanephric mesenchyme contains multipotent stem cells whose fate is restricted after induction. *Development,* 114**,** 565-72.

HEYMAN, S. N., EVANS, R. G., ROSEN, S. & ROSENBERGER, C. 2012. Cellular adaptive changes in AKI: mitigating renal hypoxic injury. *Nephrol Dial Transplant,* 27**,** 1721-8.

HEYMAN, S. N., ROSEN, S., DARMON, D., GOLDFARB, M., BITZ, H., SHINA, A. & BREZIS, M. 2000. Endotoxin-induced renal failure. II. A role for tubular hypoxic damage. *Exp Nephrol,* 8**,** 275-82.

HEYMAN, S. N., ROSENBERGER, C. & ROSEN, S. 2010. Experimental ischemia-reperfusion: biases and myths-the proximal vs. distal hypoxic tubular injury debate revisited. *Kidney Int,* 77**,** 9-16.

HIRANO, A., SHIMIZU, T., IMAMURA, H., WATANABE, O., KINOSHITA, J., OKABE, T., KIMURA, K., KAMIMURA, M., DOMOTO, K., AIBA, M. & OGAWA, K. 2006. The combination of epirubicin plus docetaxel as neoadjuvant chemotherapy in locally-advanced breast cancer. *Anticancer Res,* 26**,** 581-4.

HOCKEL, M. & VAUPEL, P. 2001. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst,* 93**,** 266-76.

HOSTE, E. A., CLERMONT, G., KERSTEN, A., VENKATARAMAN, R., ANGUS, D. C., DE BACQUER, D. & KELLUM, J. A. 2006. RIFLE criteria for acute kidney injury are associated with hospital mortality in critically ill patients: a cohort analysis. *Crit Care,* 10**,** R73.

HOSTE, E. A. & KELLUM, J. A. 2006. Acute kidney injury: epidemiology and diagnostic criteria. *Curr Opin Crit Care,* 12**,** 531-7.

HSU, C. Y., CHERTOW, G. M., MCCULLOCH, C. E., FAN, D., ORDONEZ, J. D. & GO, A. S. 2009. Nonrecovery of kidney function and death after acute on chronic renal failure. *Clin J Am Soc Nephrol,* 4**,** 891-8.

HSU, C. Y., MCCULLOCH, C. E., FAN, D., ORDONEZ, J. D., CHERTOW, G. M. & GO, A. S. 2007. Community-based incidence of acute renal failure. *Kidney Int,* 72**,** 208-12.

HSU, C. Y., ORDONEZ, J. D., CHERTOW, G. M., FAN, D., MCCULLOCH, C. E. & GO, A. S. 2008. The risk of acute renal failure in patients with chronic kidney disease. *Kidney Int,* 74**,** 101-7.

HUANG, J., LI, L., LIAN, J., SCHAUER, S., VESELY, P. W., KRATKY, D., HOEFLER, G. & LEHNER, R. 2016. Tumor-Induced Hyperlipidemia Contributes to Tumor Growth. *Cell Rep,* 15**,** 336-48.

HUANG, Y., YAN, C. H. & FU, S. B. 2005. [The cloning and expression of apoptosis associated gene ANNEXIN A2 induced by p53 gene]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi,* 22**,** 661-4.

HUDES G, C. M. T. P. D. J. F. R. K. A. S. E. S. J. M. D. B. I. K. Z. L. V. S.-W. & GLOBAL, A. T. 2007. Temsirolimus, interferon alfa, or both for advanced renal-cell carcinoma. *N Engl J Med.,* 2007**,** 2271-81.

HUFF, V. 1998. Wilms tumor genetics. *Am J Med Genet,* 79**,** 260-7.

HUI-STICKLE, S., BREWER, E. D. & GOLDSTEIN, S. L. 2005. Pediatric ARF epidemiology at a tertiary care center from 1999 to 2001. *Am J Kidney Dis,* 45**,** 96-101.

IHTIYAR, E., YASAR, N. F., ERKASAP, N., KOKEN, T., TOSUN, M., ONER, S. & ERKASAP, S. 2011. Effects of doxycycline on renal ischemia reperfusion injury induced by abdominal compartment syndrome. *J Surg Res,* 167**,** 113-20.

ILIOPOULOS, O., KIBEL, A., GRAY, S. & KAELIN, W. G., JR. 1995. Tumour suppression by the human von Hippel-Lindau gene product. *Nat Med,* 1**,** 822-6.

ILIOPOULOS, O., LEVY, A. P., JIANG, C., KAELIN, W. G., JR. & GOLDBERG, M. A. 1996. Negative regulation of hypoxia-inducible genes by the von Hippel-Lindau protein. *Proc Natl Acad Sci U S A,* 93**,** 10595-9.

ISHANI, A., XUE, J. L., HIMMELFARB, J., EGGERS, P. W., KIMMEL, P. L., MOLITORIS, B. A. & COLLINS, A. J. 2009. Acute kidney injury increases risk of ESRD among elderly. *J Am Soc Nephrol,* 20**,** 223-8.

JAAKKOLA, P., MOLE, D. R., TIAN, Y. M., WILSON, M. I., GIELBERT, J., GASKELL, S. J., VON KRIEGSHEIM, A., HEBESTREIT, H. F., MUKHERJI, M., SCHOFIELD, C. J., MAXWELL, P. H., PUGH, C. W. & RATCLIFFE, P. J. 2001. Targeting of HIF-alpha to the von Hippel-Lindau ubiquitylation complex by O2-regulated prolyl hydroxylation. *Science,* 292**,** 468-72.

JACOBSEN, J., RASMUSON, T., GRANKVIST, K. & LJUNGBERG, B. 2000. Vascular endothelial growth factor as prognostic factor in renal cell carcinoma. *J Urol,* 163**,** 343-7.

JAIN, M., ARVANITIS, C., CHU, K., DEWEY, W., LEONHARDT, E., TRINH, M., SUNDBERG, C. D., BISHOP, J. M. & FELSHER, D. W. 2002. Sustained loss of a neoplastic phenotype by brief inactivation of MYC. *Science,* 297**,** 102-4.

JANG, H. R., KO, G. J., WASOWSKA, B. A. & RABB, H. 2009. The interaction between ischemia-reperfusion and immune responses in the kidney. *J Mol Med (Berl),* 87**,** 859-64.

JANG, H. R. & RABB, H. 2015. Immune cells in experimental acute kidney injury. Nat. Rev. Nephro, 11, 88–101.

JHA, V., MALHOTRA, H. S., SAKHUJA, V. & CHUGH, K. S. 1992. Spectrum of hospital-acquired acute renal failure in the developing countries--Chandigarh study. *Q J Med,* 83**,** 497-505.

JHA, V. & PARAMESWARAN, S. 2013. Community-acquired acute kidney injury in tropical countries. *Nature reviews. Nephrology,* 9**,** 278-90.

JIA, J. W., LI, K. L., WU, J. X. & GUO, S. L. 2013. Clinical significance of annexin II expression in human non-small cell lung cancer. *Tumour Biol,* 34**,** 1767-71.

JIANG, W. G., DAVIES, G., MARTIN, T. A., PARR, C., WATKINS, G., MASON, M. D. & MANSEL, R. E. 2006. Expression of membrane type-1 matrix metalloproteinase, MT1-MMP in human breast cancer and its impact on invasiveness of breast cancer cells. *Int J Mol Med,* 17**,** 583-90.

JO, S. K., BAJWA, A., AWAD, A. S., LYNCH, K. R. & OKUSA, M. D. 2008. Sphingosine-1-phosphate receptors: biology and therapeutic potential in kidney disease. *Kidney Int,* 73**,** 1220-30.

JOHNSON, R. J., SEGAL, M. S., SRINIVAS, T., EJAZ, A., MU, W., RONCAL, C., SANCHEZ-LOZADA, L. G., GERSCH, M., RODRIGUEZ-ITURBE, B., KANG, D. H. & ACOSTA, J. H. 2005. Essential hypertension, progressive renal disease, and uric acid: a pathogenetic link? *J Am Soc Nephrol,* 16**,** 1909-19.

JONES, A. L., HULETT, M. D. & PARISH, C. R. 2005. Histidine-rich glycoprotein: A novel adaptor protein in plasma that modulates the immune, vascular and coagulation systems. *Immunol Cell Biol,* 83**,** 106-18.

JONES, R. G. & THOMPSON, C. B. 2009. Tumor suppressors and cell metabolism: A recipe for cancer growth.

JUAREZ, J. C., GUAN, X., SHIPULINA, N. V., PLUNKETT, M. L., PARRY, G. C., SHAW, D. E., ZHANG, J. C., RABBANI, S. A., MCCRAE, K. R., MAZAR, A. P., MORGAN, W. T. & DONATE, F. 2002. Histidine-proline-rich glycoprotein has potent antiangiogenic activity mediated through the histidine-proline-rich domain. *Cancer Res,* 62**,** 5344-50.

KAELIN, W. G., JR. 2007. The von Hippel-Lindau tumor suppressor protein and clear cell renal carcinoma. *Clin Cancer Res,* 13**,** 680s-684s.

KALE, S., KARIHALOO, A., CLARK, P. R., KASHGARIAN, M., KRAUSE, D. S. & CANTLEY, L. G. 2003. Bone marrow stem cells contribute to repair of the ischemically injured renal tubule. *J Clin Invest,* 112**,** 42-9.

KANIKOWSKA, D., SUGENOYA, J., SATO, M., SHIMIZU, Y., INUKAI Y., NISHIMURA N. & IWASE. S. 2010. Influence of season on plasma antidiuretic hormone, angiotensin II, aldosterone and plasma renin activity in young volunteers. *Int J Biometeorol,* 54, 243-248.

KASHYAP, V., REZENDE, N. C., SCOTLAND, K. B., SHAFFER, S.M., PERSSON, J. L., GUDAS, L. J. & MONGAN, N. P. 2009. Regulation of Stem Cell Pluripotency and Differentiation Involves a Mutual Regulatory Circuit of the Nanog, OCT4, and SOX2 Pluripotency Transcription Factors With Polycomb Repressive Complexes and Stem Cell microRNAs. *Stem Cells Dev.* 18, 193-1106.

KASSOUF, W., APRIKIAN, A. G., LAPLANTE, M. & TANGUAY, S. 2004. Natural history of renal masses followed expectantly. *J Urol,* 171**,** 111-3; discussion 113.

KATE, R. J., PEREZ, R. M., MAZUMDAR, D., PASUPATHY, K. S. & NILAKANTAN, V. 2016. Prediction and detection models for acute kidney injury in hospitalized older adults. BMC *Medl Info and Deci Making*. 16, 39.

KATO, M., SUZUKI, T., SUZUKI, Y., TERASAWA, Y., SASANO, H. & ARAI, Y. 2004. Natural history of small renal cell carcinoma: evaluation of growth rate, histological grade, cell proliferation and apoptosis. *J Urol,* 172**,** 863-866.

KAVOLIUS, J. P., MASTORAKOS, D. P., PAVLOVICH, C., RUSSO, P., BURT, M. E. & BRADY, M. S. 1998. Resection of metastatic renal cell carcinoma. *J Clin Oncol,* 16**,** 2261-6.

KEITH, B., JOHNSON, R. S. & SIMON, M. C. 2012. HIF1alpha and HIF2alpha: sibling rivalry in hypoxic tumour growth and progression. *Nat Rev Cancer,* 12**,** 9-22.

KELLUM, J. A. & CHAWLA, L. S. 2016. Cell-cycle arrest and acute kidney injury: the light and the dark sides. *Nephrol Dial Transplant,* 31**,** 16-22.

KELLUM, J. A., LEVIN, N., BOUMAN, C. & LAMEIRE, N. 2002. Developing a consensus classification system for acute renal failure. *Curr Opin Crit Care,* 8**,** 509-14.

KELLUM, J. A., UNRUH, M. L. & MURUGAN, R. 2011. Acute kidney injury. *BMJ Clin Evid,* 2011.

KERR M, B. B. M. J. O. D. D. J. M. B. 2012. Estimating the financial cost of chronic kidney disease to the NHS in England. *Nephrol Dial Transplant,* 27**,** 73-80.

KERR, M., BEDFORD, M., MATTHEWS, B. & O'DONOGHUE, D. 2014. The economic impact of acute kidney injury in England. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association,* 29**,** 1362-8.

KERR, M., BRAY, B., MEDCALF, J., O'DONOGHUE, D. J. & MATTHEWS, B. 2012. Estimating the financial cost of chronic kidney disease to the NHS in England. *Nephrol Dial Transplant,* 27 Suppl 3**,** iii73-80.

KESSLER, P. M., VASAVADA, S. P., RACKLEY, R. R., STACKHOUSE, T., DUH, F. M., LATIF, F., LERMAN, M. I., ZBAR, B. & WILLIAMS, B. R. 1995. Expression of the Von Hippel-Lindau tumor suppressor gene, VHL, in human fetal kidney and during mouse embryogenesis. *Mol Med,* 1**,** 457-66.

KHOSLA, N., SOROKO, S. B., CHERTOW, G. M., HIMMELFARB, J., IKIZLER, T. A., PAGANINI, E. & MEHTA, R. L. 2009. Preexisting chronic kidney disease: a potential for improved outcomes from acute kidney injury. *Clin J Am Soc Nephrol,* 4**,** 1914-9.

KILLION, D., CANFIELD, C., NORMAN, J. & ROSENTHAL, J. T. 1993. Exogenous epidermal growth factor fails to accelerate functional recovery in the autotransplanted ischemic pig kidney. *J Urol,* 150**,** 1551-6.

KIM, H. L., SELIGSON, D., LIU, X., JANZEN, N., BUI, M. H., YU, H., SHI, T., BELLDEGRUN, A. S., HORVATH, S. & FIGLIN, R. A. 2005. Using tumor markers to predict the survival of patients with metastatic renal cell carcinoma. *J Urol,* 173**,** 1496-501.

KIM, W. Y. & KAELIN, W. G. 2004. Role of VHL gene mutation in human cancer. *J Clin Oncol,* 22**,** 4991-5004.

KINCH, L., GRISHIN, N. V. & BRUGAROLAS, J. 2011. Succination of Keap1 and Activation of Nrf2-Dependent Antioxidant Pathways in FH-Deficient Papillary Renal Cell Carcinoma Type 2.

KIRK, A. D. 2003. Crossing the bridge: large animal models in translational transplantation research. *Immunol Rev,* 196**,** 176-96.

KITAMURA, S., YAMASAKI, Y., KINOMURA, M., SUGAYA, T., SUGIYAMA, H., MAESHIMA, Y. & MAKINO, H. 2005. Establishment and characterization of renal progenitor like cells from S3 segment of nephron in rat adult kidney. *FASEB J,* 19**,** 1789-97.

KLATTE, T., PANTUCK, A., SAID, J., SELIGSON, D., RAO, N., LAROCHELLE, J., SHUCH, B., ZISMAN, A., KABBINAVAR, F. & BELLDEGRUN, A. 2009. Cytogenetic and molecular tumor profiling for type 1 and type 2 papillary renal cell carcinoma. *Clin Cancer Res,* 15**,** 1162-1169.

KLATTE, T., SELIGSON, D. B., RIGGS, S. B., LEPPERT, J. T., BERKMAN, M. K., KLEID, M. D., YU, H., KABBINAVAR, F. F., PANTUCK, A. J. & BELLDEGRUN, A. S. 2007. Hypoxia-inducible factor 1 alpha in clear cell renal cell carcinoma. *Clin Cancer Res,* 13**,** 7388-93.

KNOEPFLER, P. S. 2007. Myc goes global: new tricks for an old oncogene. *Cancer Res,* 67**,** 5061-3.

KOBAYASHI, A., VALERIUS, M. T., MUGFORD, J. W., CARROLL, T. J., SELF, M., OLIVER, G. & MCMAHON, A. P. 2008. Six2 defines and regulates a multipotent self-renewing nephron progenitor population throughout mammalian kidney development. *Cell Stem Cell,* 3**,** 169-81.

KOLHE NV, S. P. E. C. A. V. L. G. W. H. D. A. 2008. Case mix, outcome and activity for patients with severe acute kidney injury during the first 24 hours after admission to an adult, general critical care unit: application of predictive models from a secondary analysis of the ICNARC Case Mix Programme data. *Crit Care,* 12.

KON-NO, H., ISHII, G., NAGAI, K., YOSHIDA, J., NISHIMURA, M., NARA, M., FUJII, T., MURATA, Y., MIYAMOTO, H. & OCHIAI, A. 2006. Carbonic anhydrase IX expression is associated with tumor progression and a poor prognosis of lung adenocarcinoma. *Lung Cancer,* 54**,** 409-18.

KONDO, K. & KAELIN, W. G., JR. 2001. The von Hippel-Lindau tumor suppressor gene. *Exp Cell Res,* 264**,** 117-25.

KORKMAZ, A. & KOLANKAYA, D. 2009. The protective effects of ascorbic acid against renal ischemia-reperfusion injury in male rats. *Ren Fail,* 31**,** 36-43.

KOSHIJI, M., TO, K. K., HAMMER, S., KUMAMOTO, K., HARRIS, A. L., MODRICH, P. & HUANG, L. E. 2005. HIF-1alpha induces genetic instability by transcriptionally downregulating MutSalpha expression. *Mol Cell,* 17**,** 793-803.

KOUKOURAKIS, M. I., GIATROMANOLAKI, A., SIVRIDIS, E., BOUGIOUKAS, G., DIDILIS, V., GATTER, K. C. & HARRIS, A. L. 2003. Lactate dehydrogenase-5 (LDH-5) overexpression in non-small-cell lung cancer tissues is linked to tumour hypoxia, angiogenic factor production and poor prognosis. *Br J Cancer,* 89**,** 877-85.

KOUMENIS, C., ALARCON, R., HAMMOND, E., SUTPHIN, P., HOFFMAN, W., MURPHY, M., DERR, J., TAYA, Y., LOWE, S. W., KASTAN, M. & GIACCIA, A. 2001. Regulation of p53 by hypoxia: dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. *Mol Cell Biol,* 21**,** 1297-310.

KOVACS, G., AKHTAR, M., BECKWITH, B. J., BUGERT, P., COOPER, C. S., DELAHUNT, B., EBLE, J. N., FLEMING, S., LJUNGBERG, B., MEDEIROS, L. J., MOCH, H., REUTER, V. E., RITZ, E., ROOS, G., SCHMIDT, D., SRIGLEY, J. R., STORKEL, S., VAN DEN BERG, E. & ZBAR, B. 1997a. The Heidelberg classification of renal cell tumours. *J Pathol,* 183**,** 131-3.

KOVACS, G., ERLANDSSON, R., BOLDOG, F., INGVARSSON, S., MULLER-BRECHLIN, R., KLEIN, G. & SUMEGI, J. 1988. Consistent chromosome 3p deletion and loss of heterozygosity in renal cell carcinoma. *Proc Natl Acad Sci U S A,* 85**,** 1571-5.

KOVACS, K., GIANNINI, C., SCHEITHAUER, B. W., STEFANEANU, L., LLOYD, R. V. & HORVATH, E. 1997b. Pituitary Changes in Ataxia-Telangiectasia Syndrome: An Immunocytochemical, In Situ Hybridization, and DNA Cytometric Study of Three Cases. *Endocr Pathol,* 8**,** 195-203.

KOYNER, J. L., GARG, A. X., COCA, S. G., SINT, K., THIESSEN-PHILBROOK, H., PATEL, U. D., SHLIPAK, M. G. & PARIKH, C. R. 2012. Biomarkers predict progression of acute kidney injury after cardiac surgery. *J Am Soc Nephrol,* 23**,** 905-14.

KOYNER, J. L., VAIDYA, V. S., BENNETT, M. R., MA, Q., WORCESTER, E., AKHTER, S. A., RAMAN, J., JEEVANANDAM, V., O'CONNOR, M. F., DEVARAJAN, P., BONVENTRE, J. V. & MURRAY, P. T. 2010. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol,* 5**,** 2154-65.

KRAWCZESKI, C. D., GOLDSTEIN, S. L., WOO, J. G., WANG, Y., PIYAPHANEE, N., MA, Q., BENNETT, M. & DEVARAJAN, P. 2011. Temporal relationship and predictive value of urinary acute kidney injury biomarkers after pediatric cardiopulmonary bypass. *J Am Coll Cardiol,* 58**,** 2301-9.

KRIEG, M., HAAS, R., BRAUCH, H., ACKER, T., FLAMME, I. & PLATE, K. H. 2000. Up-regulation of hypoxia-inducible factors HIF-1alpha and HIF-2alpha under normoxic conditions in renal carcinoma cells by von Hippel-Lindau tumor suppressor gene loss of function. *Oncogene,* 19**,** 5435-43.

KROEGER, N., SELIGSON, D. B., SIGNORETTI, S., YU, H., MAGYAR, C. E., HUANG, J., BELLDEGRUN, A. S. & PANTUCK, A. J. 2014. Poor prognosis and advanced clinicopathological features of clear cell renal cell carcinoma

(ccRCC) are associated with cytoplasmic subcellular localisation of Hypoxia inducible factor-2alpha. *Eur J Cancer,* 50**,** 1531-40.

KUCUK, A., KABADERE, S., TOSUN, M., KOKEN, T., KINACI, M. K., ISIKLI, B. & ERKASAP, N. 2009. Protective effects of doxycycline in ischemia/reperfusion injury on kidney. *J Physiol Biochem,* 65**,** 183-91.

KUSCHERT, S., ROWITCH, D. H., HAENIG, B., MCMAHON, A. P. & KISPERT, A. 2001. Characterization of Pax-2 regulatory sequences that direct transgene expression in the Wolffian duct and its derivatives. *Developmental biology,* 229**,** 128-40.

LAFRANCE, J. P., DJURDJEV, O. & LEVIN, A. 2010. Incidence and outcomes of acute kidney injury in a referred chronic kidney disease cohort. *Nephrol Dial Transplant,* 25**,** 2203-9.

LAHOTI, A., NATES, J. L., WAKEFIELD, C. D., PRICE, K. J. & SALAHUDEEN, A. K. 2011. Costs and outcomes of acute kidney injury in critically ill patients with cancer. *J Support Oncol,* 9**,** 149-155.

LAM, J. S., LEPPERT, J. T., FIGLIN, R. A. & BELLDEGRUN, A. S. 2005. Role of molecular markers in the diagnosis and therapy of renal cell carcinoma. *Urology,* 66**,** 1-9.

LAMB-WHARTON, R. J. & MORGAN, W. T. 1993. Induction of T-lymphocyte adhesion by histidine-proline-rich glycoprotein and concanavalin A. *Cell Immunol,* 152**,** 544-55.

LANGE, C., TOGEL, F., ITTRICH, H., CLAYTON, F., NOLTE-ERNSTING, C., ZANDER, A. R. & WESTENFELDER, C. 2005. Administered mesenchymal stem cells enhance recovery from ischemia/reperfusion-induced acute renal failure in rats. *Kidney Int,* 68**,** 1613-7.

LANGMEAD, B., SCHATZ, M. C., LIN, J., POP, M. & SALZBERG, S. L. 2009. Searching for SNPs with cloud computing. *Genome Biol,* 10**,** R134.

LANIGAN, D., CONROY, R., BARRY-WALSH, C., LOFTUS, B., ROYSTON, D. & LEADER, M. 1994. A comparative analysis of grading systems in renal adenocarcinoma. *Histopathology,* 24**,** 473-476.

LATIF, F., TORY, K., GNARRA, J., YAO, M., DUH, F. M., ORCUTT, M. L., STACKHOUSE, T., KUZMIN, I., MODI, W., GEIL, L. & ET AL. 1993. Identification of the von Hippel-Lindau disease tumor suppressor gene. *Science,* 260**,** 1317-20.

LEE, K., QIAN, D. Z., REY, S., WEI, H., LIU, J. O. & SEMENZA, G. L. 2009. Anthracycline chemotherapy inhibits HIF-1 transcriptional activity and tumor-induced mobilization of circulating angiogenic cells. *Proc Natl Acad Sci U S A,* 106**,** 2353-8.

LENBURG, M. E., LIOU, L. S., GERRY, N. P., FRAMPTON, G. M., COHEN, H. T. & CHRISTMAN, M. F. 2003. Previously unidentified changes in renal cell carcinoma gene expression identified by parametric analysis of microarray data. *BMC Cancer,* 3**,** 31.

LEO, C., GIACCIA, A. J. & DENKO, N. C. 2004. The hypoxic tumor microenvironment and gene expression. *Semin Radiat Oncol,* 14**,** 207-14.

LEUNG, K. C. W., TONELLI, M. & JAMES, M. T. 2012. Chronic kidney disease following acute kidney injury-risk and outcomes. *Nature reviews. Nephrology***,** 1-9.

LEUNG, L., SAIGO, K. & GRANT, D. 1989. Heparin binds to human monocytes and modulates their procoagulant activities and secretory phenotypes. Effects of histidine-rich glycoprotein. *Blood,* 73**,** 177-84.

LI, H. & DURBIN, R. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics,* 25**,** 1754-60.

LI, H. & DURBIN, R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics,* 26**,** 589-95.

LI, L. & KAELIN, W. G., JR. 2011. New insights into the biology of renal cell carcinoma. *Hematol Oncol Clin North Am,* 25**,** 667-86.

LI, P. K. T., BURDMANN, E. A. & MEHTA, R. L. 2013. Acute Kidney Injury: a global alert. *Jornal Brasileiro de Nefrologia,* 35**,** 1-5.

LI, S.-Y., CHEN, J.-Y., YANG, W.-C. & CHUANG, C.-L. 2011. Acute kidney injury network classification predicts in-hospital and long-term mortality in patients undergoing elective coronary artery bypass grafting surgery☆. *European Journal of Cardio-Thoracic Surgery,* 39**,** 323-328.

LIAO, D. & JOHNSON, R. S. 2007. Hypoxia: a key regulator of angiogenesis in cancer. *Cancer Metastasis Rev,* 26**,** 281-90.

LIANO, F. & PASCUAL J. 1996.Epidemiology of acute renal failure: a prospective, multicenter, community-based study. Madrid Acute Renal Failure Study Group. Kidney Int, 50(3), 811-818.

LIDGREN, A., HEDBERG, Y., GRANKVIST, K., RASMUSON, T., VASKO, J. & LJUNGBERG, B. 2005. The expression of hypoxia-inducible factor 1alpha is a favorable independent prognostic factor in renal cell carcinoma. *Clin Cancer Res,* 11**,** 1129-35.

LIEBERTHAL, W. & NIGAM, S. K. 2000. Acute renal failure. II. Experimental models of acute renal failure: imperfect but indispensable. *Am J Physiol Renal Physiol,* 278**,** F1-F12.

LIJNEN, H. R., VAN HOEF, B. & COLLEN, D. 1983. Interaction of heparin with histidine-rich glycoprotein and with antithrombin III. *Thromb Haemost,* 50**,** 560-2.

LIN, F., MORAN, A. & IGARASHI, P. 2005. Intrarenal cells, not bone marrow-derived cells, are the major source for regeneration in postischemic kidney. *J Clin Invest,* 115**,** 1756-64.

LIN, P., WANG, H. J., LEE, H., LEE, H. S., WANG, S. L., HSUEH, Y. M., TSAI, K. J. & CHEN, C. Y. 1999. NAD(P)H: quinone oxidoreductase polymorphism and lung cancer in Taiwan. *J Toxicol Environ Health A,* 58**,** 187-97.

LINDGREN, D., BOSTROM, A. K., NILSSON, K., HANSSON, J., SJOLUND, J., MOLLER, C., JIRSTROM, K., NILSSON, E., LANDBERG, G., AXELSON, H. & JOHANSSON, M. E. 2011. Isolation and characterization of progenitor-like cells from human renal proximal tubules. *Am J Pathol,* 178**,** 828-37.

LINEHAN, W. M., LERMAN, M. I. & ZBAR, B. 1995. Identification of the von Hippel-Lindau (VHL) gene. Its role in renal cancer. *JAMA,* 273**,** 564-70.

LINEHAN, W. M., PINTO, P. A., BRATSLAVSKY, G., PFAFFENROTH, E., MERINO, M., VOCKE, C. D., TORO, J. R., BOTTARO, D., NECKERS, L., SCHMIDT, L. S. & SRINIVASAN, R. Hereditary kidney cancer: Unique opportunity for disease-based therapy. 2009. 2252-2261.

LINEHAN, W. M., PINTO, P. A., BRATSLAVSKY, G., PFAFFENROTH, E., MERINO, M., VOCKE, C. D., TORO, J. R., BOTTARO, D., NECKERS, L., SCHMIDT, L. S. & SRINIVASAN, R. Hereditary kidney cancer: Unique opportunity for disease-based therapy. 2009 2009. 2252-2261.

LINEHAN, W. M., PINTO, P. A., SRINIVASAN, R., MERINO, M., CHOYKE, P., CHOYKE, L., COLEMAN, J., TORO, J., GLENN, G., VOCKE, C., ZBAR, B., SCHMIDT, L. S., BOTTARO, D. & NECKERS, L. 2007. Identification of

the genes for kidney cancer: opportunity for disease-specific targeted therapeutics. *Clin Cancer Res,* 13**,** 671s-679s.

LINEHAN, W. M. & RICKETTS, C. J. 2013. The metabolic basis of kidney cancer. *Semin Cancer Biol,* 23**,** 46-55.

LINEHAN, W. M., SRINIVASAN, R. & SCHMIDT, L. S. 2010. The genetic basis of kidney cancer: a metabolic disease. *Nature reviews. Urology,* 7**,** 277-285.

LING, Q., JACOVINA, A. T., DEORA, A., FEBBRAIO, M., SIMANTOV, R., SILVERSTEIN, R. L., HEMPSTEAD, B., MARK, W. H. & HAJJAR, K. A. 2004. Annexin II regulates fibrin homeostasis and neoangiogenesis in vivo. *J Clin Invest,* 113**,** 38-48.

LITTLE, M. H. 2006. Regrow or repair: potential regenerative therapies for the kidney. *J Am Soc Nephrol,* 17**,** 2390-401.

LITTLE, M. H. & MCMAHON, A. P. 2012. Mammalian kidney development: principles, progress, and projections. *Cold Spring Harb Perspect Biol,* 4.

LIU, G., CHERESH, P. & KAMP, D. W. 2013. Molecular basis of asbestos-induced lung disease. *Annu Rev Pathol,* 8**,** 161-87.

LIU, Y., YAN, S., JI, C., DAI, W., HU, W., et al. 2012.Metabolomic changes and protective effect of (L)-carnitine in rat kidney ischemia/reperfusion injury. Kidney Blood Press Res, 35: 373–381.

LJUNGKVIST, A. S., BUSSINK, J., KAANDERS, J. H. & VAN DER KOGEL, A. J. 2007. Dynamics of tumor hypoxia measured with bioreductive hypoxic cell markers. *Radiat Res,* 167**,** 127-45.

LO, L. J., GO, A. S., CHERTOW, G. M., MCCULLOCH, C. E., FAN, D., ORDONEZ, J. D. & HSU, C. Y. 2009. Dialysis-requiring acute renal failure increases the risk of progressive chronic kidney disease. *Kidney Int,* 76**,** 893-9.

LOKMAN, N. A., WEEN, M. P., OEHLER, M. K. & RICCIARDELLI, C. 2011. The role of annexin A2 in tumorigenesis and cancer progression. *Cancer Microenviron,* 4**,** 199-208.

LOUIE, S. M., ROBERTS, L. S., MULVIHILL, M. M., LUO, K. & NOMURA, D. K. 2013. Cancer cells incorporate and remodel exogenous palmitate into structural and oncogenic signaling lipids. *Biochim Biophys Acta,* 1831**,** 1566-72.

LU, C. Y. 2014. beta-Lapachone ameliorates murine cisplatin nephrotoxicity: NAD(+), NQO1, and SIRT1 at the crossroads of metabolism, injury, and inflammation. *Kidney Int,* 85**,** 496-8.

LU, H., YANG, Z., ZHANG, H., GAN, M., ZHOU, T. & WANG, S. 2013. The expression and clinical significance of matrix metalloproteinase 7 and tissue inhibitor of matrix metalloproteinases 2 in clear cell renal cell carcinoma. *Exp Ther Med,* 5**,** 890-896.

LUBENSKY, I. A., GNARRA, J. R., BERTHEAU, P., WALTHER, M. M., LINEHAN, W. M. & ZHUANG, Z. 1996. Allelic deletions of the VHL gene detected in multiple microscopic clear cell renal lesions in von Hippel-Lindau disease patients. *Am J Pathol,* 149**,** 2089-94.

LUNNEY, J. K. 2007. Advances in swine biomedical model genomics. *Int J Biol Sci,* 3**,** 179-84.

MA, Y., KONG, J., YAN, G., REN, X., JIN, D., JIN, T., LIN, L. & LIN, Z. 2014. NQO1 overexpression is associated with poor prognosis in squamous cell carcinoma of the uterine cervix. *BMC Cancer,* 14**,** 414.

MABJEESH, N. J., AVIDOR, Y. & MATZKIN, H. 2004. Emerging nephron sparing treatments for kidney tumors: a continuum of modalities from energy ablation to laparoscopic partial nephrectomy. *The Journal of urology,* 171**,** 553-60.

MACHERET, M. & HALAZONETIS, T. D. 2015. DNA replication stress as a hallmark of cancer. *Annu Rev Pathol,* 10**,** 425-48.

MAESHIMA, A., MAESHIMA, K., NOJIMA, Y. & KOJIMA, I. 2002. Involvement of Pax-2 in the action of activin A on tubular cell regeneration. *J Am Soc Nephrol,* 13**,** 2850-9.

MAESHIMA, A., SAKURAI, H. & NIGAM, S. K. 2006. Adult kidney tubular cell population showing phenotypic plasticity, tubulogenic capacity, and integration capability into developing kidney. *J Am Soc Nephrol,* 17**,** 188-98.

MAESHIMA, A., YAMASHITA, S. & NOJIMA, Y. 2003. Identification of renal progenitor-like tubular cells that participate in the regeneration processes of the kidney. *J Am Soc Nephrol,* 14**,** 3138-46.

MAHER, E. R. & KAELIN, W. G., JR. 1997. von Hippel-Lindau disease. *Medicine (Baltimore),* 76**,** 381-91.

MAI, J., FINLEY, R. L., JR., WAISMAN, D. M. & SLOANE, B. F. 2000. Human procathepsin B interacts with the annexin II tetramer on the surface of tumor cells. *J Biol Chem,* 275**,** 12806-12.

MALEK, M. & NEMATBAKHSH, M. 2015. Renal ischemia/reperfusion injury; from pathophysiology to treatment. *J Renal Inj Prev,* 4**,** 20-7.

MANDELBAUM, T., SCOTT, D. J., LEE, J., MARK, R. G., MALHOTRA, A., WAIKAR, S. S., HOWELL, M. D. & TALMOR, D. 2011. Outcome of critically ill patients with acute kidney injury using the Acute Kidney Injury Network criteria. *Critical care medicine,* 39**,** 2659-64.

MASCHIETTO, M., TRAPE, A. P., PICCOLI, F. S., RICCA, T. I., DIAS, A. A., COUDRY, R. A., GALANTE, P. A., TORRES, C., FAHHAN, L., LOURENCO, S., GRUNDY, P. E., DE CAMARGO, B., DE SOUZA, S., NEVES, E. J., SOARES, F. A., BRENTANI, H. & CARRARO, D. M. 2011. Temporal blastemal cell gene expression analysis in the kidney reveals new Wnt and related signaling pathway genes to be essential for Wilms' tumor onset. *Cell Death Dis,* 2**,** e224.

MATSUNAGA, T., INABA, T., MATSUI, H., OKUYA, M., MIYAJIMA, A., INUKAI, T., FUNABIKI, T., ENDO, M., LOOK, A. T. & KUROSAWA, H. 2004. Regulation of annexin II by cytokine-initiated signaling pathways and E2A-HLF oncoprotein. *Blood,* 103**,** 3185-91.

MAXWELL, P. H., PUGH, C. W. & RATCLIFFE, P. J. 2001. Activation of the HIF pathway in cancer. *Curr Opin Genet Dev,* 11**,** 293-9.

MAXWELL, P. H., WIESENER, M. S., CHANG, G. W., CLIFFORD, S. C., VAUX, E. C., COCKMAN, M. E., WYKOFF, C. C., PUGH, C. W., MAHER, E. R. & RATCLIFFE, P. J. 1999. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. *Nature,* 399**,** 271-5.

MCLAREN, W., GIL, L., HUNT, S. E., RIAT, H. S., RITCHIE, G. R., THORMANN, A., FLICEK, P. & CUNNINGHAM, F. 2016. The Ensembl Variant Effect Predictor. *Genome Biol,* 17**,** 122.

MCLAUGHLIN, N., YAO, X., LI, SAIFUDEEN, Y., Z. & EL-DAHR, S. S. 2013. Histone signature of metanephric mesenchyme cell lines. *Epigenetics*, 8(9), 970–978

MEGYESI, J., PRICE, P. M., TAMAYO, E. & SAFIRSTEIN, R. L. 1999. The lack of a functional p21(WAF1/CIP1) gene ameliorates progression to chronic renal failure. *Proc Natl Acad Sci U S A,* 96**,** 10830-5.

MEHTA, R. L. & CHERTOW, G. M. 2003. Acute renal failure definitions and classification: time for change? *J Am Soc Nephrol,* 14**,** 2178-87.

MEHTA, R. L., KELLUM, J. A., SHAH, S. V., MOLITORIS, B. A., RONCO, C., WARNOCK, D. G. & LEVIN, A. 2007. Acute Kidney Injury Network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care,* 11**,** R31.

MEHTA, R.L., PASCUAL, M.T., SOROKO, S., SAVAGE, B. R., HIMMELFARB, J., IKIZLER, T. A., PAGANINI, E. P. & CHERTOW, G.M. 2004. Program to Improve Care in Acute Renal Disease. Kidney Int., 66(4), 1613-21.

MENELL, J. S., CESARMAN, G. M., JACOVINA, A. T., MCLAUGHLIN, M. A., LEV, E. A. & HAJJAR, K. A. 1999. Annexin II and bleeding in acute promyelocytic leukemia. *N Engl J Med,* 340**,** 994-1004.

METNITZ, P. G. H., KRENN, C. G., STELTZER, H., LANG, T., PLODER, J., LENZ, K., LE GALL, J.-R. & DRUML, W. 2002. Effect of acute renal failure requiring renal replacement therapy on outcome in critically ill patients. *Critical care medicine,* 30**,** 2051-8.

MIERAU, G. W., BECKWITH, J. B. & WEEKS, D. A. 1987. Ultrastructure and histogenesis of the renal tumors of childhood: an overview. *Ultrastruct Pathol,* 11**,** 313-33.

MILLER, F., KENTSIS, A., OSMAN, R. & PAN, Z. Q. 2005. Inactivation of VHL by tumorigenic mutations that disrupt dynamic coupling of the pVHL.hypoxia-inducible transcription factor-1alpha complex. *J Biol Chem,* 280**,** 7985-96.

MINTON, D. R., FU, L., CHEN, Q., ROBINSON, B. D., GROSS, S. S., NANUS, D. M. & GUDAS, L. J. 2015. Analyses of the transcriptome and metabolome demonstrate that HIF1alpha mediates altered tumor metabolism in clear cell renal cell carcinoma. *PLoS One,* 10**,** e0120649.

MIRZAZADEH M. & RICHARDS, K. A 2011. Complete Duplication of Collecting System in a Horseshoe Kidney Presenting with Recurrent Urinary Tract Infections: Report of an Exceedingly Rare Congenital Anomaly and Review of Literature. The sci world J, 11, 1591–1596.

MIYAZAKI, Y., SHIMIZU, A., ICHIKAWA, I., HOSOYA, T., PASTAN, I. & MATSUSAKA, T. 2014. Mice are unable to endogenously regenerate podocytes during the repair of immunotoxin-induced glomerular injury. *Nephrol Dial Transplant,* 29**,** 1005-12.

MOELLER, B. J., RICHARDSON, R. A. & DEWHIRST, M. W. 2007. Hypoxia and radiotherapy: opportunities for improved outcomes in cancer treatment. *Cancer Metastasis Rev,* 26**,** 241-8.

MOHAMMAD, H. S., KUROKOHCHI, K., YONEYAMA, H., TOKUDA, M., MORISHITA, A., JIAN, G., SHI, L., MUROTA, M., TANI, J., KATO, K., MIYOSHI, H., DEGUCHI, A., HIMOTO, T., USUKI, H., WAKABAYASHI, H., IZUISHI, K., SUZUKI, Y., IWAMA, H., DEGUCHI, K., UCHIDA, N., SABET, E. A., ARAFA, U. A., HASSAN, A. T., EL-SAYED, A. A. & MASAKI, T. 2008. Annexin A2 expression and phosphorylation are up-regulated in hepatocellular carcinoma. *Int J Oncol,* 33**,** 1157-63.

MOREL, E. & GRUENBERG, J. 2007. The p11/S100A10 light chain of annexin A2 is dispensable for annexin A2 association to endosomes and functions in endosomal transport. *PLoS One,* 2**,** e1118.

MORI, S., SHINOHATA, R., RENBUTSU, M., TAKAHASHI, H. K., FANG, Y. I., YAMAOKA, K., OKAMOTO, M., YAMAMOTO, I. & NISHIBORI, M. 2003. Histidine-rich glycoprotein plus zinc reverses growth inhibition of vascular smooth muscle cells by heparin. *Cell Tissue Res,* 312**,** 353-9.

MORRISON, S. J., WHITE, P. M., ZOCK, C. & ANDERSON, D. J. 1999. Prospective identification, isolation by flow cytometry, and in vivo self-renewal of multipotent mammalian neural crest stem cells. *Cell,* 96**,** 737-49.

MOTZER, R. J., HUTSON, T. E., CELLA, D., REEVES, J., HAWKINS, R., GUO, J., NATHAN, P., STAEHLER, M., DE SOUZA, P., MERCHAN, J. R., BOLETI, E., FIFE, K., JIN, J., JONES, R., UEMURA, H., DE GIORGI, U., HARMENBERG, U., WANG, J., STERNBERG, C. N., DEEN, K., MCCANN, L., HACKSHAW, M. D., CRESCENZO, R., PANDITE, L. N. & CHOUEIRI, T. K. 2013. Pazopanib versus sunitinib in metastatic renal-cell carcinoma. *The New England journal of medicine,* 369**,** 722-31.

MOULDER, J. E. & ROCKWELL, S. 1987. Tumor hypoxia: its impact on cancer therapy. *Cancer Metastasis Rev,* 5**,** 313-41.

MURLIMANJU, B. V., KUMAR, B. M., KUMAR, N., PRASHANTH, K. U., RAO, C. P., GURU, A., PRABHU, L. V. & KUMAR,C. G. 2014. Morphometric parameters of the human adult kidney: An anatomical study. Int. J. Morphol., 32(2), 656-659.

NAGASAKI, Y., YOSHITOMI , T. &HIRAYAMA, A. 2011. Acute kidney injury - Experimental models novel redox nanotherapy for renal ischemia reperfusion injury. ndt Plus, 4 (2), 4.

NAGASE, H. & WOESSNER, J. F., JR. 1999. Matrix metalloproteinases. *J Biol Chem,* 274**,** 21491-4.

NAITO, M., BOMSZTYK, K. & ZAGER, R. A. 2009. Renal ischemia-induced cholesterol loading: transcription factor recruitment and chromatin remodeling along the HMG CoA reductase gene. *Am J Pathol,* 174**,** 54-62.

NAKAGAWA, T., SASAHARA, M., HANEDA, M., KATAOKA, H., NAKAGAWA, H., YAGI, M., KIKKAWA, R. & HAZAMA, F. 1999. Role of PDGF B-chain and PDGF receptors in rat tubular regeneration after acute injury. *Am J Pathol,* 155**,** 1689-99.

NAKATAKE, Y., FUKUI, N., IWAMATSU, Y., MASUI, S., TAKAHASHI, K. YAGI, R.,YAGI, K.,MIYAZAKI, JUN-ICHI., MATOBA, R., KO, M. S. H. & NIWA, H. 2006. Klf4 Cooperates with Oct3/4 and Sox2 To Activate the Lefty1 Core Promoter in Embryonic Stem Cells. Mol Cellular Bio, 26, 7772–7782.

NANGAKU, M. 2006. Chronic hypoxia and tubulointerstitial injury: a final common pathway to end-stage renal failure. *J Am Soc Nephrol,* 17**,** 17-25.

NASH, K., HAFEEZ, A. & HOU, S. 2002. Hospital-acquired renal insufficiency. *Am J Kidney Dis,* 39**,** 930-6.

NATH, K. A. 1992. Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis,* 20**,** 1-17.

NAUDIN TEN CATE, L., VERMEIJ-KEERS, C., SMIT, D. A., COHEN-OVERBEEK, T. E., GERSSEN-SCHOORL, K. B. & DIJKHUIZEN, T. 1995. Intracranial teratoma with multiple fetuses: pre- and post-natal appearance. *Hum Pathol,* 26**,** 804-7.

NESBIT, C. E., TERSAK, J. M. & PROCHOWNIK, E. V. 1999. MYC oncogenes and human neoplastic disease. *Oncogene,* 18**,** 3004-16.

NGUYEN, S. & HSU, C. Y. 2007. Excess weight as a risk factor for kidney failure. *Curr Opin Nephrol Hypertens,* 16**,** 71-6.

NIELSEN, P. M., ELDIRDIRI,A., BERTELSEN, L. B., JORGENSEN, H. S., ARDENKJAER-LARSEN, J. H. & LAUSTSEN, C. 2016. Fumarase activity: an in vivo and in vitro biomarker for acute kidney injury. Sci Reports, 7, 40812.

NIKUMBH, R. D., KAZI S. & UGHADE, M. N. 2014. Gross anatomy of the horseshoe kidney: a case report with brief review of literature. Eur J Anat, 18 (2), 128-131.

NIN, N., LOMBARDI, R., FRUTOS-VIVAR, F., ESTEBAN, A., LORENTE, J. A., FERGUSON, N. D., HURTADO, J., APEZTEGUÍA, C., BROCHARD, L., SCHORTGEN, F., RAYMONDOS, K., TOMICIC, V., SOTO, L., GONZÁLEZ, M., NIGHTINGALE, P., ABROUG, F., PELOSI, P., ARABI, Y., MORENO, R. & ANZUETO, A. 2010. EARLY AND SMALL CHANGES IN SERUM CREATININE CONCENTRATIONS ARE ASSOCIATED WITH MORTALITY IN MECHANICALLY VENTILATED PATIENTS. *Shock,* 34**,** 109-116.

NIOI, P. & HAYES, J. D. 2004. Contribution of NAD(P)H:quinone oxidoreductase 1 to protection against carcinogenesis, and regulation of its gene by the Nrf2 basic-region leucine zipper and the arylhydrocarbon receptor basic helix-loop-helix transcription factors. *Mutat Res,* 555**,** 149-71.

NISSENSON, A. R. 1998. Acute renal failure: definition and pathogenesis. *Kidney Int Suppl,* 66**,** S7-10.

OH, E.-T. & PARK, H. J. 2015. Implications of NQO1 in cancer therapy. *BMB Reports,* 48**,** 609-617.

OHNO, Y., IZUMI, M., KAWAMURA, T., NISHIMURA, T., MUKAI, K. & TACHIBANA, M. 2009. Annexin II represents metastatic potential in clear-cell renal cell carcinoma. *Br J Cancer,* 101**,** 287-94.

OKUSA, M. D. 2002. A(2A) adenosine receptor: a novel therapeutic target in renal disease. *Am J Physiol Renal Physiol,* 282**,** F10-8.

OKUSA, M. D., CHERTOW, G. M. & PORTILLA, D. 2009. The nexus of acute kidney injury, chronic kidney disease, and World Kidney Day 2009. *Clin J Am Soc Nephrol,* 4**,** 520-2.

OLENCHOCK, B. A., MOSLEHI, J., BAIK, A. H., DAVIDSON, S. M., WILLIAMS, J., GIBSON, W. J., CHAKRABORTY, A. A., PIERCE, K. A., MILLER, C. M., HANSE, E. A., KELEKAR, A., SULLIVAN, L. B., WAGERS, A. J., CLISH, C. B., VANDER HEIDEN, M. G. & KAELIN, W. G., JR. 2016. EGLN1 Inhibition and Rerouting of alpha-Ketoglutarate Suffice for Remote Ischemic Protection. *Cell,* 165**,** 497.

OLIFERENKO, S., PAIHA, K., HARDER, T., GERKE, V., SCHWARZLER, C., SCHWARZ, H., BEUG, H., GUNTHERT, U. & HUBER, L. A. 1999. Analysis of CD44-containing lipid rafts: Recruitment of annexin II and stabilization by the actin cytoskeleton. *J Cell Biol,* 146**,** 843-54.

OLIVER, J. A., BARASCH, J., YANG, J., HERZLINGER, D. & AL-AWQATI, Q. 2002. Metanephric mesenchyme contains embryonic renal stem cells. *Am J Physiol Renal Physiol,* 283**,** F799-809.

OLIVER, J. A., MAAROUF, O., CHEEMA, F. H., MARTENS, T. P. & AL-AWQATI, Q. 2004. The renal papilla is a niche for adult kidney stem cells. *J Clin Invest,* 114**,** 795-804.

OLSEN, H. M., PARISH, C. R. & ALTIN, J. G. 1996. Histidine-rich glycoprotein binding to T-cell lines and its effect on T-cell substratum adhesion is strongly potentiated by zinc. *Immunology,* 88**,** 198-206.

OLSSON, A. K., LARSSON, H., DIXELIUS, J., JOHANSSON, I., LEE, C., OELLIG, C., BJORK, I. & CLAESSON-WELSH, L. 2004. A fragment of histidine-rich glycoprotein is a potent inhibitor of tumor vascularization. *Cancer Res,* 64**,** 599-605.

OOI, A., WONG, J. C., PETILLO, D., ROOSSIEN, D., PERRIER-TRUDOVA, V., WHITTEN, D., MIN, B. W. H., TAN, M. H., ZHANG, Z., YANG, X. J., ZHOU, M., GARDIE, B., MOLINI, V., RICHARD, S. P., TAN, P. H., TEH, B. T. & FURGE, K. A. 2011. An Antioxidant Response Phenotype Shared between Hereditary and Sporadic Type 2 Papillary Renal Cell Carcinoma. *Cancer Cell,* 20**,** 511-523.

OUDARD, S., GEORGE, D., MEDIONI, J. & MOTZER, R. 2007. Treatment options in renal cell carcinoma: past, present and future. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO,* 18 Suppl 1**,** x25-31.

PAGES, G. & POUYSSEGUR, J. 2005. Transcriptional regulation of the Vascular Endothelial Growth Factor gene--a concert of activating factors. *Cardiovasc Res,* 65**,** 564-73.

PAJIC, A., SPITKOVSKY, D., CHRISTOPH, B., KEMPKES, B., SCHUHMACHER, M., STAEGE, M. S., BRIELMEIER, M., ELLWART, J., KOHLHUBER, F., BORNKAMM, G. W., POLACK, A. & EICK, D. 2000. Cell cycle activation by c-myc in a burkitt lymphoma model cell line. *Int J Cancer,* 87**,** 787-93.

PALMER L. G. & SCHNERMANN, J. 2015.Integrated Control of Na Transport along the Nephron. *Clin J Am Soc Nephrol,* 10(4): 676–687.

PALMIERI, T., LAVRENTIEVA, A. & GREENHALGH, D. 2009. An assessment of acute kidney injury with modified RIFLE criteria in pediatric patients with severe burns. *Intensive Care Med,* 35**,** 2125-9.

PALMIERI, T., LAVRENTIEVA, A. & GREENHALGH, D. G. 2010. Acute kidney injury in critically ill burn patients. Risk factors, progression and impact on mortality. *Burns,* 36**,** 205-11.

PANNU, N. & NADIM, M. K. 2008. An overview of drug-induced acute kidney injury. *Crit Care Med,* 36**,** S216-23.

PANTUCK, A. J., ZENG, G., BELLDEGRUN, A. S. & FIGLIN, R. A. 2003. Pathobiology, prognosis, and targeted therapy for renal cell carcinoma: exploiting the hypoxia-induced pathway. *Clin Cancer Res,* 9**,** 4641-52.

PANTUCK, A. J., ZISMAN, A. & BELLDEGRUN, A. S. 2001. The changing natural history of renal cell carcinoma. *The Journal of urology,* 166**,** 1611-1623.

PARFREY, P. S., GRIFFITHS, S. M., BARRETT, B. J., PAUL, M. D., GENGE, M., WITHERS, J., FARID, N. & MCMANAMON, P. J. 1989. Contrast material-induced renal failure in patients with diabetes mellitus, renal insufficiency, or both. A prospective controlled study. *N Engl J Med,* 320**,** 143-9.

PARIKH, A. & SHAW, A. 2012. The Economics of Renal Failure and Kidney Disease in Critically Ill Patients.

PARIKH, C. R., DEVARAJAN, P., ZAPPITELLI, M., SINT, K., THIESSEN-PHILBROOK, H., LI, S., KIM, R. W., KOYNER, J. L., COCA, S. G., EDELSTEIN, C. L., SHLIPAK, M. G., GARG, A. X. & KRAWCZESKI, C. D. 2011. Postoperative biomarkers predict acute kidney injury and poor outcomes after pediatric cardiac surgery. *J Am Soc Nephrol,* 22**,** 1737-47.

PARIKH, C. R., EDELSTEIN, C. L., DEVARAJAN, P. & CANTLEY, L. 2007. Biomarkers of acute kidney injury: early diagnosis, pathogenesis, and recovery. *J Investig Med,* 55**,** 333-40.

PARK, K. M., CHEN, A. & BONVENTRE, J. V. 2001. Prevention of kidney ischemia/reperfusion-induced functional injury and JNK, p38, and MAPK kinase activation by remote ischemic pretreatment. *J Biol Chem,* 276**,** 11870-6.

PATARD, J. J., RIOUX-LECLERCQ, N. & FERGELOT, P. 2006. Understanding the importance of smart drugs in renal cell carcinoma.

PATEL, P. H., CHADALAVADA, R. S. V., CHAGANTI, R. S. K. & MOTZER, R. J. 2006. Targeting von Hippel-Lindau pathway in renal cell carcinoma. *Clinical Cancer Research,* 12**,** 7215-7220.

PATEL S. K. R. & DRESSLER, G. R. 2013.The Genetics and Epigenetics of Kidney Development. Semin Nephrol, 33(4), 314–326.

PATRAKI, E. & CARDILLO, M. R. 2007. Quantitative immunohistochemical analysis of matrilysin 1 (MMP-7) in various renal cell carcinoma subtypes. *Int J Immunopathol Pharmacol,* 20**,** 697-705.

PAVLOVICH, C. P. & SCHMIDT, L. S. 2004. Searching for the hereditary causes of renal-cell carcinoma. *Nat Rev Cancer,* 4**,** 381-93.

PEREIRA-SAMPAIO, M. A., FAVORITO, L.A. & SAMPAIO, F.J. 2004. Pig kidney: anatomical relationships between the intrarenal arteries and the kidney collecting system. Applied study for urological research and surgical training. J Urol, 172(5 Pt 1), 2077-2081.

PELENGARIS, S., KHAN, M. & EVAN, G. 2002. c-MYC: more than just a matter of life and death. *Nat Rev Cancer,* 2**,** 764-76.

PHILLIPS, J. L., PAVLOVICH, C. P., WALTHER, M., RIED, T. & LINEHAN, W. M. 2001. The genetic basis of renal epithelial tumors: advances in research and its impact on prognosis and therapy. *Curr Opin Urol,* 11**,** 463-9.

PIGNOT, G., ELIE, C., CONQUY, S., VIEILLEFOND, A., FLAM, T., ZERBIB, M., DEBRE, B. & AMSELLEM-OUAZANA, D. 2007. Survival analysis of 130 patients with papillary renal cell carcinoma: prognostic utility of type 1 and type 2 subclassification. *Urology,* 69**,** 230-5.

PIKOR, L., THU, K., VUCIC, E. & LAM, W. 2013. The detection and implication of genome instability in cancer. *Cancer Metastasis Rev,* 32**,** 341-52.

PINO, C. J. & HUMES, H. D. 2010. Stem cell technology for the treatment of acute and chronic renal failure. *Transl Res,* 156**,** 161-8.

PIRES, I. M., BENCOKOVA, Z., MILANI, M., FOLKES, L. K., LI, J. L., STRATFORD, M. R., HARRIS, A. L. & HAMMOND, E. M. 2010. Effects of acute versus chronic hypoxia on DNA damage responses and genomic instability. *Cancer Res,* 70**,** 925-35.

PISONI, R., WILLE, K. M. & TOLWANI, A. J. 2008. The epidemiology of severe acute kidney injury: from BEST to PICARD, in acute kidney injury: new concepts. *Nephron Clin Pract,* 109**,** c188-91.

PLEBANI, M. & PIVA, E. 2002. Erythrocyte sedimentation rate: use of fresh blood for quality control. *Am J Clin Pathol,* 117**,** 621-6.

PODE-SHAKKED, N. & DEKEL, B. 2011. Wilms tumor--a renal stem cell malignancy? *Pediatr Nephrol,* 26**,** 1535-43.

POLYAK, K. & HAHN, W. C. 2006. Roots and stems: stem cells in cancer. *Nat Med,* 12**,** 296-300.

PONTE, B., FELIPE, C., MURIEL, A., TENORIO, M. T. & LIANO, F. 2008. Long-term functional evolution after an acute kidney injury: a 10-year study. *Nephrol Dial Transplant,* 23**,** 3859-66.

PORE, N., JIANG, Z., GUPTA, A., CERNIGLIA, G., KAO, G. D. & MAITY, A. 2006. EGFR tyrosine kinase inhibitors decrease VEGF expression by both hypoxia-inducible factor (HIF)-1-independent and HIF-1-dependent mechanisms. *Cancer Res,* 66**,** 3197-204.

POULSOM, R., FORBES, S. J., HODIVALA-DILKE, K., RYAN, E., WYLES, S., NAVARATNARASAH, S., JEFFERY, R., HUNT, T., ALISON, M., COOK, T., PUSEY, C. & WRIGHT, N. A. 2001. Bone marrow contributes to renal parenchymal turnover and regeneration. *J Pathol,* 195**,** 229-35.

PRABHAKAR, N. R. & SEMENZA, G. L. 2012. Adaptive and maladaptive cardiorespiratory responses to continuous and intermittent hypoxia mediated by hypoxia-inducible factors 1 and 2. *Physiol Rev,* 92**,** 967-1003.

PRALL, O. W., ROGAN, E. M., MUSGROVE, E. A., WATTS, C. K. & SUTHERLAND, R. L. 1998. c-Myc or cyclin D1 mimics estrogen effects on cyclin E-Cdk2 activation and cell cycle reentry. *Mol Cell Biol,* 18**,** 4499-508.

PRESCOTT, L. F. 1966. The normal urinary excretion rates of renal tubular cells, leucocytes and red blood cells. *Clin Sci,* 31**,** 425-35.

PRITCHETT, T. L., BADER, H. L., HENDERSON J. AND HSU. T. 2015. Conditional inactivation of the mouse von Hippel–Lindau tumor suppressor gene results in wide-spread hyperplastic, inflammatory and fibrotic lesions in the kidney. *Oncogene,* 34, 2631-2639

PUPCA, G., MICLAUS, G. D., BUCURAS, V., IACOB, N., SAS, I., MATUSZ, P., TUBBS, R. S. & LOUKAS, M. 2014.Left crossed fused renal ectopia L-shaped kidney type, with double nutcracker syndrome (anterior and posterior). Rom J Morphol Embryol, 55(3 Suppl), 1237–1241.

Qiao, J., Cohen, D. & Herzlinger. D. 1995. The metanephric blastema differentiates into collecting system and nephron epithelia in vitro. *Development,* 121, 3207-3214.

QI, Y., TU, Y., YANG, D., CHEN, Q., XIAO, J., CHEN, Y., FU, J., XIAO, X. & ZHOU, Z. 2007. Cyclin A but not cyclin D1 is essential for c-myc-modulated cell-cycle progression. *J Cell Physiol,* 210**,** 63-71.

QUAGGIN, S. E. & KREIDBERG, J. A. 2008. Development of the renal glomerulus: good neighbors and good fences. *Development (Cambridge, England),* 135**,** 609-20.

QUINLAN, A. R. & HALL, I. M. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. *Bioinformatics,* 26**,** 841-2.

RAGHAVENDRA, MALLIKARJUN, & VIDYA, M. J. 2013.Functions of kidney & artificial kidneys. Intern Jl Innov Res In Elect, Elect, Instrum & Control Engin, 1(1), 1-5.

RAKHRA, K., BACHIREDDY, P., ZABUAWALA, T., ZEISER, R., XU, L., KOPELMAN, A., FAN, A. C., YANG, Q., BRAUNSTEIN, L., CROSBY, E., RYEOM, S. & FELSHER, D. W. 2010. CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. *Cancer Cell,* 18**,** 485-98.

RAMESH, G..& RANGANATHAN P. 2014. Mouse Models and Methods for Studying Human Disease, Acute Kidney Injury (AKI). Mouse Gen, 1194 (Methods in Molecular Biology), 421-436.

RANKIN, E. B., TOMASZEWSKI, J. E. & HAASE, V. H. 2006. Renal Cyst Development in Mice with Conditional Inactivation of the von Hippel-Lindau Tumor Suppressor. Cancer Res, 66, 5.

RAO K, E. V. & BATTULA S. Rao 2015. Horseshoe kidney: a review article. Int J Res Med Sci, 3(11), 3004-3007.

RAZORENOVA, O. V., CASTELLINI, L., COLAVITTI, R., EDGINGTON, L. E., NICOLAU, M., HUANG, X., BEDOGNI, B., MILLS, E. M., BOGYO, M. & GIACCIA, A. J. 2014. The apoptosis repressor with a CARD domain (ARC) gene is a direct hypoxia-inducible factor 1 target gene and promotes survival and proliferation of VHL-deficient renal cancer cells. *Mol Cell Biol,* 34**,** 739-51.

REMUZZI, G., BENIGNI, A. & REMUZZI, A. 2006. Mechanisms of progression and regression of renal lesions of chronic nephropathies and diabetes. *J Clin Invest,* 116**,** 288-96.

RENBAUM, P., DUH, F. M., LATIF, F., ZBAR, B., LERMAN, M. I. & KUZMIN, I. 1996. Isolation and characterization of the full-length 3' untranslated region of the human von Hippel-Lindau tumor suppressor gene. *Hum Genet,* 98**,** 666-71.

REYA, T., MORRISON, S. J., CLARKE, M. F. & WEISSMAN, I. L. 2001. Stem cells, cancer, and cancer stem cells. *Nature,* 414**,** 105-11.

RICHARDS, F. M., SCHOFIELD, P. N., FLEMING, S. & MAHER, E. R. 1996. Expression of the von Hippel-Lindau disease tumour suppressor gene during human embryogenesis. *Hum Mol Genet,* 5**,** 639-44.

RINKEVICH, Y., MONTORO, D. T., CONTRERAS-TRUJILLO, H., HARARI-STEINBERG, O., NEWMAN, A. M., TSAI, J. M., LIM, X., VAN-AMERONGEN, R., BOWMAN, A., JANUSZYK, M., PLENICEANU, O., NUSSE, R., LONGAKER, M. T., WEISSMAN, I. L. & DEKEL, B. 2014. In vivo clonal analysis reveals lineage-restricted progenitor characteristics in mammalian kidney development, maintenance, and regeneration. *Cell Rep,* 7**,** 1270-83.

RIVA, C., CHAUVIN, C., PISON, C. & LEVERVE, X. 1998. Cellular physiology and molecular events in hypoxia-induced apoptosis. *Anticancer Res,* 18**,** 4729-36.

ROBERTS, D. S., HAYCOCK, G. B., DALTON, R. N., TURNER, C., TOMLINSON, P., STIMMLER, L. & SCOPES, J. W. 1990. Prediction of acute renal failure after birth asphyxia. *Arch Dis Child,* 65**,** 1021-8.

ROBSON CJ, C. B. M. A. W. 1969. The results of radical nephrectomy for renal cell carcinoma. *J Urol.,* 101**,** 297-301.

RODRIGO, J. P., LEQUERICA-FERNANDEZ, P., ROSADO, P., ALLONCA, E., GARCIA-PEDRERO, J. M. & DE VICENTE, J. C. 2011. Clinical significance of annexin A2 downregulation in oral squamous cell carcinoma. *Head Neck,* 33**,** 1708-14.

RONCONI, E., SAGRINATI, C., ANGELOTTI, M. L., LAZZERI, E., MAZZINGHI, B., BALLERINI, L., PARENTE, E., BECHERUCCI, F., GACCI, M., CARINI, M., MAGGI, E., SERIO, M., VANNELLI, G. B., LASAGNI, L., ROMAGNANI, S. & ROMAGNANI, P. 2009. Regeneration of glomerular podocytes by human renal progenitors. *J Am Soc Nephrol,* 20**,** 322-32.

ROSEMAN, B. J., BOLLEN, A., HSU, J., LAMBORN, K. & ISRAEL, M. A. 1994. Annexin II marks astrocytic brain tumors of high histologic grade. *Oncol Res,* 6**,** 561-7.

ROSENBERGER, C., MANDRIOTA, S., JURGENSEN, J. S., WIESENER, M. S., HORSTRUP, J. H., FREI, U., RATCLIFFE, P. J., MAXWELL, P. H., BACHMANN, S. & ECKARDT, K. U. 2002. Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. *J Am Soc Nephrol,* 13**,** 1721-32.

ROSENBERGER, C., ROSEN, S. & HEYMAN, S. N. 2006. Renal parenchymal oxygenation and hypoxia adaptation in acute kidney injury. *Clin Exp Pharmacol Physiol,* 33**,** 980-8.

ROY, S., BISWAS, S., KHANNA, S., GORDILLO, G., BERGDALL, V., GREEN, J., MARSH, C. B., GOULD, L. J. & SEN, C. K. 2009. Characterization of a preclinical model of chronic ischemic wound. *Physiol Genomics,* 37**,** 211-24.

RUSSO, P. 2000. Renal cell carcinoma: presentation, staging, and surgical treatment.

SAFIRSTEIN, R. 1999. Renal regeneration: reiterating a developmental paradigm. *Kidney Int,* 56**,** 1599-600.

SAFIRSTEIN, R., PRICE, P. M., SAGGI, S. J. & HARRIS, R. C. 1990. Changes in gene expression after temporary renal ischemia. *Kidney Int,* 37**,** 1515-21.

SALAMA ME, G. K. S. H. P. E. P. J. M. M. A. M. B. D. P.-V. M. 2005. pT1 substaging in renal cell carcinoma: validation of the 2002 TNM staging modification of malignant renal epithelial tumors. *J Urol.,* 173**,** 1492-5.

SALLUSTIO, F., DE BENEDICTIS, L., CASTELLANO, G., ZAZA, G., LOVERRE, A., COSTANTINO, V., GRANDALIANO, G. & SCHENA, F. P. 2010. TLR2 plays a role in the activation of human resident renal stem/progenitor cells. *Faseb j,* 24**,** 514-25.

SAMPAIO, F. J., PEREIRA-SAMPAIO, M. A. & FAVORITO, L. A. 1998.The pig kidney as an endourologic model: anatomic contribution. J Endourol, 12, 45.

SANDLUND, J., OOSTERWIJK, E., GRANKVIST, K., OOSTERWIJK-WAKKA, J., LJUNGBERG, B. & RASMUSON, T. 2007. Prognostic impact of carbonic anhydrase IX expression in human renal cell carcinoma. *BJU Int,* 100**,** 556-60.

SANTONI-RUGIU, E., FALCK, J., MAILAND, N., BARTEK, J. & LUKAS, J. 2000. Involvement of Myc activity in a G(1)/S-promoting mechanism parallel to the pRb/E2F pathway. *Mol Cell Biol,* 20**,** 3497-509.

SARAFIAN, T., PRADEL, L. A., HENRY, J. P., AUNIS, D. & BADER, M. F. 1991. The participation of annexin II (calpactin I) in calcium-evoked exocytosis requires protein kinase C. *J Cell Biol,* 114**,** 1135-47.

SATO, Y., YOSHIZATO, T., SHIRAISHI, Y., MAEKAWA, S., OKUNO, Y., KAMURA, T., SHIMAMURA, T., SATO-OTSUBO, A., NAGAE, G., SUZUKI, H., NAGATA, Y., YOSHIDA, K., KON, A., SUZUKI, Y., CHIBA, K., TANAKA, H., NIIDA, A., FUJIMOTO, A., TSUNODA, T., MORIKAWA, T., MAEDA, D., KUME, H., SUGANO, S., FUKAYAMA, M., ABURATANI, H., SANADA, M., MIYANO, S., HOMMA, Y. & OGAWA, S. 2013. Integrated molecular analysis of clear-cell renal cell carcinoma. *Nature genetics,* 45**,** 860-7.

SAXEN, L. & SARIOLA, H. 1987. Early organogenesis of the kidney. *Pediatr Nephrol,* 1**,** 385-92.

SCHATZ, M. C. 2009. CloudBurst: highly sensitive read mapping with MapReduce. *Bioinformatics,* 25**,** 1363-9.

SCHLONDORFF, D. O. 2008. Overview of factors contributing to the pathophysiology of progressive renal disease. *Kidney Int,* 74**,** 860-6.

SCHMID, T., ZHOU, J. & BRUNE, B. 2004. HIF-1 and p53: communication of transcription factors under hypoxia. *J Cell Mol Med,* 8**,** 423-31.

SCHMIDT-NIELSEN, B. & O'DELL, R. 1961. Structure and concentrating mechanism in the mammalian kidney. *Am J Physiol,* 200**,** 1119-24.

SCHNEIDER, J., KHEMANI, R., GRUSHKIN, C. & BART, R. 2010. Serum creatinine as stratified in the RIFLE score for acute kidney injury is associated with mortality and length of stay for children in the pediatric intensive care unit. *Crit Care Med,* 38**,** 933-9.

SCHOKRPUR, S., HU, J., MOUGHON, D. L., LIU, P., LIN1, L. C., HERMANN, K., MANGUL, S., GUAN, W., PELLEGRINI, M., XU, H. & WU, L. 2016. CRISPR-Mediated VHL Knockout Generates an Improved Model for Metastatic Renal Cell Carcinoma. Sci Reports, 6, 29032.

SCHOOK, L. B., BEEVER, J. E., ROGERS, J., HUMPHRAY, S., ARCHIBALD, A., CHARDON, P., MILAN, D., ROHRER, G. & EVERSOLE, K. 2005. Swine Genome Sequencing Consortium (SGSC): a strategic roadmap for sequencing the pig genome. *Comp Funct Genomics,* 6**,** 251-5.

SCHRIER, R. W., WANG, W., POOLE, B. & MITRA, A. 2004. Acute renal failure: definitions, diagnosis, pathogenesis, and therapy. *J Clin Invest,* 114**,** 5-14.

SCHWAB, K., PATTERSON, L. T., ARONOW, B. J., HUNG-CHI LIANG, R. & POTTER, S. S. 2003. A catalogue of gene expression in the developing kidney. Kidney Int. 64, 1588–1604.

SCORSETTI, M., FACOETTI, A., NAVARRIA, P., BIGNARDI, M., DE SANTIS, M., NINONE, S. A., LATTUADA, P., URSO, G., VIGORITO, S., MANCOSU, P. & DEL VECCHIO, M. 2009. Hypofractionated stereotactic radiotherapy and radiosurgery for the treatment of patients with radioresistant brain metastases. *Anticancer Research,* 29**,** 4259-4263.

SEHIC, D., KARLSSON, J., SANDSTEDT, B. & GISSELSSON, D. 2012. SIX1 protein expression selectively identifies blastemal elements in Wilms tumor. *Pediatr Blood Cancer,* 59**,** 62-8.

SEIZINGER, B. R., ROULEAU, G. A., OZELIUS, L. J., LANE, A. H., FARMER, G. E., LAMIELL, J. M., HAINES, J., YUEN, J. W., COLLINS, D., MAJOOR-KRAKAUER, D. & ET AL. 1988. Von Hippel-Lindau disease maps to the region of chromosome 3 associated with renal cell carcinoma. *Nature,* 332**,** 268-9.

SELF, M., LAGUTIN, O. V., BOWLING, B., HENDRIX, J., CAI, Y., DRESSLER, G. R. & OLIVER, G. 2006. Six2 is required for suppression of nephrogenesis and progenitor renewal in the developing kidney. *Embo j,* 25**,** 5214-28.

SEMENZA, G. L. 2002a. HIF-1 and tumor progression: pathophysiology and therapeutics. *Trends Mol Med,* 8**,** S62-7.

SEMENZA, G. L. 2002b. Involvement of hypoxia-inducible factor 1 in human cancer. *Intern Med,* 41**,** 79-83.

SEMENZA, G. L. 2003. Targeting HIF-1 for cancer therapy. *Nat Rev Cancer,* 3**,** 721-32.

SENGUPTA, S., LOHSE, C. M., CHEVILLE, J. C., LEIBOVICH, B. C., THOMPSON, R. H., WEBSTER, W. S., FRANK, I., ZINCKE, H., BLUTE, M. L. & KWON, E. D. 2006. The preoperative erythrocyte sedimentation rate is an independent prognostic factor in renal cell carcinoma. *Cancer,* 106**,** 304-312.

SERMEUS, A. & MICHIELS, C. 2011. Reciprocal influence of the p53 and the hypoxic pathways. *Cell Death Dis,* 2**,** e164.

KUMAR, S., DUYVO, A., JUNQIN, FU & LI, H. 2016. Comparative assessment of methods for the fusion transcripts detection from RNA-Seq data. Sci Reports, 6, 21597.

SHARMA, M. C. & SHARMA, M. 2007. The role of annexin II in angiogenesis and tumor progression: a potential therapeutic target. *Curr Pharm Des,* 13**,** 3568-75.

SHARMA, M. R., KOLTOWSKI, L., OWNBEY, R. T., TUSZYNSKI, G. P. & SHARMA, M. C. 2006a. Angiogenesis-associated protein annexin II in breast cancer: selective expression in invasive breast cancer and contribution to tumor invasion and progression. *Exp Mol Pathol,* 81**,** 146-56.

SHARMA, M. R., ROTHMAN, V., TUSZYNSKI, G. P. & SHARMA, M. C. 2006b. Antibody-directed targeting of angiostatin's receptor annexin II inhibits Lewis Lung Carcinoma tumor growth via blocking of plasminogen activation: possible biochemical mechanism of angiostatin's action. *Exp Mol Pathol,* 81**,** 136-45.

SHUIN, T., KONDO, K., TORIGOE, S., KISHIDA, T., KUBOTA, Y., HOSAKA, M., NAGASHIMA, Y., KITAMURA, H., LATIF, F., ZBAR, B. & ET AL. 1994. Frequent somatic mutations and loss of heterozygosity of the von Hippel-Lindau tumor suppressor gene in primary human renal cell carcinomas. *Cancer Res,* 54**,** 2852-5.

SIEGEL, D., FRANKLIN, W. A. & ROSS, D. 1998. Immunohistochemical detection of NAD(P)H:quinone oxidoreductase in human lung and lung tumors. *Clin Cancer Res,* 4**,** 2065-70.

SIEGEL, R., MILLER, K. & JEMAL, A. 2015. Cancer statistics , 2015. *CA Cancer J Clin,* 65**,** 29-29.

SIEGEL, R. L., MILLER, K. D. & JEMAL, A. 2016. Cancer statistics, 2016. *CA Cancer J Clin,* 66**,** 7-30.

SILVA, P. 1990. Energy and fuel substrate metabolism in the kidney. *Semin Nephrol,* 10**,** 432-44.

SIMANTOV, R., FEBBRAIO, M., CROMBIE, R., ASCH, A. S., NACHMAN, R. L. & SILVERSTEIN, R. L. 2001. Histidine-rich glycoprotein inhibits the antiangiogenic effect of thrombospondin-1. *J Clin Invest,* 107**,** 45-52.

SIMMONS, M. N., SCHREIBER, M. J. & GILL, I. S. 2008. Surgical renal ischemia: a contemporary overview. *J Urol,* 180**,** 19-30.

SINKE, R. J., DIJKHUIZEN, T., JANSSEN, B., OLDE WEGHUIS, D., MERKX, G., VAN DEN BERG, E., SCHUURING, E., MELONI, A. M., DE JONG, B. & GEURTS VAN KESSEL, A. 1997. Fine mapping of the human renal oncocytoma-associated translocation (5;11)(q35;q13) breakpoint. *Cancer Genet Cytogenet,* 96, 95-101.

SKRYPNYK, N. I., HARRIS, R. C. & DE CAESTECKER, M. P. 2013. Ischemia-reperfusion Model of Acute Kidney Injury and Post Injury Fibrosis in Mice. *J Vis Exp,* 78, 1-6.

SM, B. 2010. Acute kidney injury: diagnosis and classification of AKI: AKIN or RIFLE? Bagshaw SM. *Nephrol., Nat Rev,* 6**,** 71-3.

SM NURUL HASSAN, B. U. A. M. A. K. A. M. K. M. K. 2013. Morphological Study of Human Kidney – A Post Mortem Study. *Bangladesh Journal of Anatomy,* 11**,** 3-6.

SNOEIJS, M. G., VAN HEURN, L. W. & BUURMAN, W. A. 2010. Biological modulation of renal ischemia-reperfusion injury. *Curr Opin Organ Transplant,* 15**,** 190-9.

SOBIN LH, G. M., WITTEKIND C 2009. *TNM classification of malignant tumors. UICC International*

Union Against Cancer., Wiley-Blackwell,.

SPIJKERS-HAGELSTEIN, J. A., MIMOSO PINHANCOS, S., SCHNEIDER, P., PIETERS, R. & STAM, R. W. 2013. Src kinase-induced phosphorylation of annexin A2 mediates glucocorticoid resistance in MLL-rearranged infant acute lymphoblastic leukemia. *Leukemia,* 27**,** 1063-71.

SPURGEON, K. R., DONOHOE, D. L. & BASILE, D. P. 2005. Transforming growth factor-beta in acute renal failure: receptor expression, effects on proliferation, cellularity, and vascularization after recovery from injury. *Am J Physiol Renal Physiol,* 288**,** F568-77.

STEC, R., GRALA, B., MACZEWSKI, M., BODNAR, L. & SZCZYLIK, C. 2009a. Chromophobe renal cell cancer--review of the literature and potential methods of treating metastatic disease. *Journal of experimental & clinical cancer research : CR,* 28**,** 134-134.

STEC, R., GRALA, B., MACZEWSKI, M., BODNAR, L. & SZCZYLIK, C. 2009b. Chromophobe renal cell cancer--review of the literature and potential methods of treating metastatic disease. *J Exp Clin Cancer Res,* 28**,** 134.

STOLLE, C., GLENN, G., ZBAR, B., HUMPHREY, J. S., CHOYKE, P., WALTHER, M., PACK, S., HURLEY, K., ANDREY, C., KLAUSNER, R. & LINEHAN, W. M. 1998. Improved detection of germline mutations in the von Hippel-Lindau disease tumor suppressor gene. *Hum Mutat,* 12**,** 417-23.

STROO, I., STOKMAN, G., TESKE, G. J., RAVEN, A., BUTTER, L. M., FLORQUIN, S. & LEEMANS, J. C. 2010. Chemokine expression in renal ischemia/reperfusion injury is most profound during the reparative phase. *Int Immunol,* 22**,** 433-42.

SULLIVAN, R. & GRAHAM, C. H. 2007. Hypoxia-driven selection of the metastatic phenotype. *Cancer Metastasis Rev,* 26**,** 319-31.

SUSANTITAPHONG, P., CRUZ, D. N., CERDA, J., ABULFARAJ, M., ALQAHTANI, F., KOULOURIDIS, I. & JABER, B. L. 2013. World incidence of AKI: A meta-analysis. *Clinical Journal of the American Society of Nephrology,* 8**,** 1482-1493.

SUTHERLAND, S. M. & ALEXANDER, S. R. 2012. Continuous renal replacement therapy in children. *Pediatr Nephrol,* 27**,** 2007-16.

SYMONS, J. M., CHUA, A. N., SOMERS, M. J., BAUM, M. A., BUNCHMAN, T. E., BENFIELD, M. R., BROPHY, P. D., BLOWEY, D., FORTENBERRY, J. D., CHAND, D., FLORES, F. X., HACKBARTH, R., ALEXANDER, S. R., MAHAN, J., MCBRYDE, K. D. & GOLDSTEIN, S. L. 2007. Demographic characteristics of pediatric continuous renal replacement therapy: a report of the prospective pediatric continuous renal replacement therapy registry. *Clin J Am Soc Nephrol,* 2**,** 732-8.

TABRIZI, G. A., BÖSE, K., REIMANN, Y. & KESSEL, M. 2013.Geminin Is Required for the Maintenance of Pluripotency. PLoS ONE, 8(9), e73826.

TANG, N., WANG, L., ESKO, J., GIORDANO, F. J., HUANG, Y., GERBER, H. P., FERRARA, N. & JOHNSON, R. S. 2004. Loss of HIF-1alpha in endothelial

cells disrupts a hypoxia-driven VEGF autocrine loop necessary for tumorigenesis. *Cancer Cell,* 6**,** 485-95.

TANG, S. W., CHANG, W. H., SU, Y. C., CHEN, Y. C., LAI, Y. H., WU, P. T., HSU, C. I., LIN, W. C., LAI, M. K. & LIN, J. Y. 2009. MYC pathway is activated in clear cell renal cell carcinoma and essential for proliferation of clear cell renal cell carcinoma cells. *Cancer Lett,* 273**,** 35-43.

TERAI, K., DONG, G. Z., OH, E. T., PARK, M. T., GU, Y., SONG, C. W. & PARK, H. J. 2009. Cisplatin enhances the anticancer effect of beta-lapachone by upregulating NQO1. *Anticancer Drugs,* 20**,** 901-9.

THADHANI, R., PASCUAL, M. & BONVENTRE, J. V. 1996. Acute renal failure. *N Engl J Med,* 334**,** 1448-60.

THAKAR, C. V., CHRISTIANSON, A., FREYBERG, R., ALMENOFF, P. & RENDER, M. L. 2009. Incidence and outcomes of acute kidney injury in intensive care units: a Veterans Administration study. *Crit Care Med,* 37**,** 2552-8.

TOGEL, F., WEISS, K., YANG, Y., HU, Z., ZHANG, P. & WESTENFELDER, C. 2007. Vasculotropic, paracrine actions of infused mesenchymal stem cells are important to the recovery from acute kidney injury. *Am J Physiol Renal Physiol,* 292**,** F1626-35.

TORRE, L. A., BRAY, F., SIEGEL, R. L., FERLAY, J., LORTET-TIEULENT, J. & JEMAL, A. 2015. Global Cancer Statistics, 2012. *CA: a cancer journal for clinicians,* 00**,** 1-22.

TRAPNELL, C., PACHTER, L. & SALZBERG, S. L. 2009. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics,* 25**,** 1105-11.

TRAPNELL, C., WILLIAMS, B. A., PERTEA, G., MORTAZAVI, A., KWAN, G., VAN BAREN, M. J., SALZBERG, S. L., WOLD, B. J. & PACHTER, L. 2010. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat Biotechnol,* 28**,** 511-5.

TRAVER, R. D., HORIKOSHI, T., DANENBERG, K. D., STADLBAUER, T. H., DANENBERG, P. V., ROSS, D. & GIBSON, N. W. 1992. NAD(P)H:quinone oxidoreductase gene expression in human colon carcinoma cells: characterization of a mutation which modulates DT-diaphorase activity and mitomycin sensitivity. *Cancer Res,* 52**,** 797-802.

TSUI, K. H., SHVARTS, O., SMITH, R. B., FIGLIN, R., DE KERNION, J. B. & BELLDEGRUN, A. 2000. Renal cell carcinoma: prognostic significance of incidentally detected tumors.

TURAJLIC, S., LARKIN, J. & SWANTON, C. 2015. SnapShot: Renal Cell Carcinoma. *Cell,* 163**,** 1556-1556e1.

TWARDOWSKI, P. W., MACK, P. C. & LARA, P. N. 2014. Papillary renal cell carcinoma: Current progress and future directions.

TZAO, C., LEE, S. C., TUNG, H. J., HSU, H. S., HSU, W. H., SUN, G. H., YU, C. P., JIN, J. S. & CHENG, Y. L. 2008. Expression of hypoxia-inducible factor (HIF)-1alpha and vascular endothelial growth factor (VEGF)-D as outcome predictors in resected esophageal squamous cell carcinoma. *Dis Markers,* 25**,** 141-8.

UCHINO, S., BELLOMO, R., GOLDSMITH, D., BATES, S. & RONCO, C. 2006. An assessment of the RIFLE criteria for acute renal failure in hospitalized patients. *Critical care medicine,* 34**,** 1913-7.

UCHINO, S., KELLUM, J. A., BELLOMO, R., DOIG, G. S., TAN, I. & BOUMAN, C. 2005. Acute renal failure in critically ill patients. *JAMA,* 294**,** 813-818.

UEHARA, T., HORINOUCHI, A., MORIKAWA, Y., TONOMURA, Y., MINAMI , K., ONO, A., YAMATE, J., YAMADA, H., OHNO, Y. URUSHIDANI, T. 2014 Identification of metabolomic biomarkers for drug-induced acute kidney injury in rats. J Appl Toxicol, 34(10),1087-1095.

UZZO, R. G. & NOVICK, A. C. 2001. Nephron sparing surgery for renal tumors: indications, techniques and outcomes. *J Urol,* 166**,** 6-18.

VACHVANICHSANONG, P., DISSANEEWATE, P., LIM, A. & MCNEIL, E. 2006. Childhood acute renal failure: 22-year experience in a university hospital in southern Thailand. *Pediatrics,* 118**,** e786-91.

VAIDYA, V. S., FERGUSON, M. A. & BONVENTRE, J. V. 2008. Biomarkers of acute kidney injury. *Annu Rev Pharmacol Toxicol,* 48**,** 463-93.

VAIDYA, V. S., SHANKAR, K., LOCK, E. A., BUCCI, T. J. & MEHENDALE, H. M. 2003a. Renal injury and repair following S-1, 2 dichlorovinyl-L-cysteine administration to mice. *Toxicol Appl Pharmacol,* 188**,** 110-21.

VAIDYA, V. S., SHANKAR, K., LOCK, E. A., BUCCI, T. J. & MEHENDALE, H. M. 2003b. Role of tissue repair in survival from s-(1,2-dichlorovinyl)-L-cysteine-induced acute renal tubular necrosis in the mouse. *Toxicol Sci,* 74**,** 215-27.

VAN DEN BERG, E., VAN DER HOUT, A. H., OOSTERHUIS, J. W., STORKEL, S., DIJKHUIZEN, T., DAM, A., ZWEERS, H. M., MENSINK, H. J., BUYS, C. H. & DE JONG, B. 1993. Cytogenetic analysis of epithelial renal-cell tumors: relationship with a new histopathological classification. *Int J Cancer,* 55**,** 223-7.

VAN DEN BERG, E., VAN OVEN, M. W., DE JONG, B., DAM, A., WIERSEMA, J., DIJKHUIZEN, T., HOEKSTRA, H. J. & MOLENAAR, W. M. 1994. Comparison of cytogenetic abnormalities and deoxyribonucleic acid ploidy of benign, borderline malignant, and different grades of malignant soft tissue tumors. *Lab Invest,* 70**,** 307-13.

VANDER , A.J., ed. 1985.Rend Phvsiology. 3" edition, Mcgraw Hill Book Co., NY, USA, 6-17.

VAN RIGGELEN, J., MULLER, J., OTTO, T., BEUGER, V., YETIL, A., CHOI, P. S., KOSAN, C., MOROY, T., FELSHER, D. W. & EILERS, M. 2010. The interaction between Myc and Miz1 is required to antagonize TGFbeta-dependent autocrine signaling during lymphoma formation and maintenance. *Genes Dev,* 24**,** 1281-94.

VARRIER, M., FORNI, L. G. & OSTERMANN, M. 2015. Long-term sequelae from acute kidney injury: potential mechanisms for the observed poor renal outcomes. *Crit Care,* 19**,** 102.

VAUPEL, P. 2004. The role of hypoxia-induced factors in tumor progression. *Oncologist,* 9 Suppl 5**,** 10-7.

VAUPEL, P., BRIEST, S. & HOCKEL, M. 2002. Hypoxia in breast cancer: pathogenesis, characterization and biological/therapeutic implications. *Wien Med Wochenschr,* 152**,** 334-42.

VAUPEL, P. & HARRISON, L. 2004. Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. *Oncologist,* 9 Suppl 5**,** 4-9.

VAUPEL, P. & MAYER, A. 2007. Hypoxia in cancer: significance and impact on clinical outcome. *Cancer Metastasis Rev,* 26**,** 225-39.

VAUPEL, P., THEWS, O. & HOECKEL, M. 2001. Treatment resistance of solid tumors: role of hypoxia and anemia. *Med Oncol,* 18**,** 243-59.

VEIKKOLA, T., KARKKAINEN, M., CLAESSON-WELSH, L. & ALITALO, K. 2000. Regulation of angiogenesis via vascular endothelial growth factor receptors. *Cancer Res,* 60**,** 203-12.

VELICKOVIC, M., DELAHUNT, B., STORKEL, S. & GREBEM, S. K. 2001. VHL and FHIT locus loss of heterozygosity is common in all renal cancer morphotypes but differs in pattern and prognostic significance. *Cancer Res,* 61**,** 4815-9.

VENKATACHALAM, M. A., GRIFFIN, K. A., LAN, R., GENG, H., SAIKUMAR, P. & BIDANI, A. K. 2010. Acute kidney injury: a springboard for progression in chronic kidney disease. *Am J Physiol Renal Physiol,* 298**,** F1078-94.

VISHWANATHA, J. K., CHIANG, Y., KUMBLE, K. D., HOLLINGSWORTH, M. A. & POUR, P. M. 1993. Enhanced expression of annexin II in human pancreatic carcinoma cells and primary pancreatic cancers. *Carcinogenesis,* 14**,** 2575-9.

VITA, M. & HENRIKSSON, M. 2006. The Myc oncoprotein as a therapeutic target for human cancer. *Semin Cancer Biol,* 16**,** 318-30.

VITOLS, S., ANGELIN, B., ERICSSON, S., GAHRTON, G., JULIUSSON, G., MASQUELIER, M., PAUL, C., PETERSON, C., RUDLING, M., SODERBERG-REID, K. & ET AL. 1990. Uptake of low density lipoproteins by human leukemic cells in vivo: relation to plasma lipoprotein levels and possible relevance for selective chemotherapy. *Proc Natl Acad Sci U S A,* 87**,** 2598-602.

VOUSDEN, K. H. & PRIVES, C. 2009. Blinded by the Light: The Growing Complexity of p53. *Cell,* 137**,** 413-31.

W., F. D. L. M. C. A. F. D. V. R. J. L. V. B. 2012. A European Renal Best Practice (ERBP) position statement on the Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines on acute kidney injury: part 1: definitions, conservative management and contrast-induced nephropathy. *Nephrol Dial Transplant.,* 27**,** 4263-72.

WAIKAR, S. S. & WINKELMAYER, W. C. 2009. Chronic on acute renal failure: long-term implications of severe acute kidney injury. *JAMA,* 302**,** 1227-9.

WALD, R., QUINN, R. R., LUO, J., LI, P., SCALES, D. C., MAMDANI, M. M. & RAY, J. G. 2009. Chronic dialysis and death among survivors of acute kidney injury requiring dialysis. *JAMA,* 302**,** 1179-85.

WALDERT, M., HAITEL, A., MARBERGER, M., KATZENBEISSER, D., OZSOY, M., STADLER, E. & REMZI, M. 2008. Comparison of type I and II papillary renal cell carcinoma (RCC) and clear cell RCC. *BJU Int,* 102**,** 1381-4.

WALTHER, M. M., LUBENSKY, I. A., VENZON, D., ZBAR, B. & LINEHAN, W. M. 1995. Prevalence of microscopic lesions in grossly normal renal parenchyma from patients with von Hippel-Lindau disease, sporadic renal cell carcinoma and no renal disease: clinical implications. *J Urol,* 154**,** 2010-4; discussion 2014-5.

WANG, C. Y., CHEN, C. L., TSENG, Y. L., FANG, Y. T., LIN, Y. S., SU, W. C., CHEN, C. C., CHANG, K. C., WANG, Y. C. & LIN, C. F. 2012a. Annexin A2 silencing induces G2 arrest of non-small cell lung cancer cells through p53-dependent and -independent mechanisms. *J Biol Chem,* 287**,** 32512-24.

WANG, C. Y. & LIN, C. F. 2014. Annexin A2: its molecular regulation and cellular expression in cancer development. *Dis Markers,* 2014**,** 308976.

WANG, H., MANNAVA, S., GRACHTCHOUK, V., ZHUANG, D., SOENGAS, M. S., GUDKOV, A. V., PROCHOWNIK, E. V. & NIKIFOROV, M. A. 2008. c-Myc depletion inhibits proliferation of human tumor cells at various stages of the cell cycle. *Oncogene,* 27**,** 1905-15.

WANG, H. E., MUNTNER, P., CHERTOW, G. M. & WARNOCK, D. G. 2012b. Acute kidney injury and mortality in hospitalized patients. *American Journal of Nephrology,* 35**,** 349-355.

WANG, K., SINGH, D., ZENG, Z., COLEMAN, S. J., HUANG, Y., SAVICH, G. L., HE, X., MIECZKOWSKI, P., GRIMM, S. A., PEROU, C. M., MACLEOD, J. N., CHIANG, D. Y., PRINS, J. F. & LIU, J. 2010a. MapSplice: accurate mapping of RNA-seq reads for splice junction discovery. *Nucleic Acids Res,* 38**,** e178.

WANG, S., ZHOU, Y., SEAVEY, C. N., SINGH, A. K., XU, X., HUNT, T., HOYT, R. F., JR. & HORVATH, K. A. 2010b. Rapid and dynamic alterations of gene expression profiles of adult porcine bone marrow-derived stem cell in response to hypoxia. *Stem Cell Res,* 4**,** 117-28.

WARD, P. S. & THOMPSON, C. B. 2012. Metabolic Reprogramming: A Cancer Hallmark Even Warburg Did Not Anticipate.

WARNOCK, D. G. 2005. Towards a definition and classification of acute kidney injury. *J Am Soc Nephrol,* 16**,** 3149-50.

WATT, F. M., FRYE, M. & BENITAH, S. A. 2008. MYC in mammalian epidermis: how can an oncogene stimulate differentiation? *Nat Rev Cancer,* 8**,** 234-42.

WEN, X., MURUGAN, R., PENG, Z. & KELLUM, J. A. 2010. Pathophysiology of acute kidney injury: a new perspective. *Contrib Nephrol,* 165**,** 39-45.

WEI, Q., XIAO, X., FOGLE P. & DONG, Z. 2014. Changes in Metabolic Profiles during Acute Kidney Injury and Recovery following Ischemia/Reperfusion. PLOS ONE . 9 (9), e106647.

Wellik, D. M., Hawkes, P. J. & Capecchi1 M. R. 2002. Hox11 paralogous genes are essential for metanephric kidney induction. Genes & Dev, 16,1423–1432.

WETTERSTEN, H. I., HAKIMI, A. A., MORIN, D., BIANCHI, C., JOHNSTONE, M. E., DONOHOE, D. R., TROTT, J. F., ABU ABOUD, O., STIRDIVANT, S., NERI, B., WOLFERT, R., STEWART, B., PEREGO, R., HSIEH, J. J. & WEISS, R. H. 2015. Grade-dependent metabolic reprogramming in kidney cancer revealed by combined proteomics and metabolomics analysis. *Cancer Research,* 75**,** 2541-2552.

WHEELER, D. S., DEVARAJAN, P., MA, Q., HARMON, K., MONACO, M., CVIJANOVICH, N. & WONG, H. R. 2008. Serum neutrophil gelatinase-associated lipocalin (NGAL) as a marker of acute kidney injury in critically ill children with septic shock. *Crit Care Med,* 36**,** 1297-303.

WILSON, D. M., 3RD & THOMPSON, L. H. 2007. Molecular mechanisms of sister-chromatid exchange. *Mutat Res,* 616**,** 11-23.

WITZGALL, R., BROWN, D., SCHWARZ, C. & BONVENTRE, J. V. 1994. Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney. Evidence for a heterogenous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. *J Clin Invest,* 93**,** 2175-88.

WOODWARD, E. R., BUCHBERGER, A., CLIFFORD, S. C., HURST, L. D., AFFARA, N. A. & MAHER, E. R. 2000. Comparative sequence analysis of the VHL tumor suppressor gene. *Genomics,* 65**,** 253-65.

WRIGHT, N. A. 2000. Epithelial stem cell repertoire in the gut: clues to the origin of cell lineages, proliferative units and cancer. *Int J Exp Pathol,* 81**,** 117-43.

WU, V. C., HUANG, T. M., LAI, C. F., SHIAO, C. C., LIN, Y. F., CHU, T. S., WU, P. C., CHAO, C. T., WANG, J. Y., KAO, T. W., YOUNG, G. H., TSAI, P. R., TSAI, H. B., WANG, C. L., WU, M. S., CHIANG, W. C., TSAI, I. J., HU, F. C., LIN, S. L., CHEN, Y. M., TSAI, T. J., KO, W. J. & WU, K. D. 2011. Acute-on-chronic kidney injury at hospital discharge is associated with long-term dialysis and mortality.[Erratum appears in Kidney Int. 2012 Apr;81(7):708]. *Kidney International,* 80**,** 1222-1230.

WU, V.C., YOUNG, G.H., HUANG, P.H., LO, S. C., WANG, K. C., SUN, C.Y., LIANG, C.J., HUANG, T.M., CHEN, J. H., CHANG, F. C., CHEN, Y. L., KUO, Y.S., CHEN, J.B., CHEN, J. W., CHEN, Y. M., KO, W. J., WU, K. D. & NSARF group. 2013. In acute kidney injury, indoxyl sulfate impairs human endothelial progenitor cells: modulation by statin. *Angiogenesis,* 16, 609–624.

XU, K., DING, Q., FANG, Z., ZHENG, J., GAO, P., LU, Y. & ZHANG, Y. 2010. Silencing of HIF-1alpha suppresses tumorigenicity of renal cell carcinoma through induction of apoptosis. *Cancer Gene Ther,* 17**,** 212-22.

XU, X. H., PAN, W., KANG, L. H., FENG, H. & SONG, Y. Q. 2015. Association of annexin A2 with cancer development (Review). *Oncol Rep,* 33**,** 2121-8.

XUE, J. L., DANIELS, F., STAR, R. A., KIMMEL, P. L., EGGERS, P. W., MOLITORIS, B. A., HIMMELFARB, J. & COLLINS, A. J. 2006. Incidence and mortality of acute renal failure in Medicare beneficiaries, 1992 to 2001. *J Am Soc Nephrol,* 17**,** 1135-42.

YAMAMURA, S., SAINI, S., MAJID, S., HIRATA, H., UENO, K., CHANG, I., TANAKA, Y., GUPTA, A. & DAHIYA, R. 2012. MicroRNA-34a suppresses malignant transformation by targeting c-Myc transcriptional complexes in human renal cell carcinoma. *Carcinogenesis,* 33**,** 294-300.

YANG, J. C., HAWORTH, L., SHERRY, R. M., HWU, P., SCHWARTZENTRUBER, D. J., TOPALIAN, S. L., STEINBERG, S. M., CHEN, H. X. & ROSENBERG, S. A. 2003. A randomized trial of bevacizumab, an anti-vascular endothelial growth factor antibody, for metastatic renal cancer. *The New England journal of medicine,* 349**,** 427-34.

YANG, Y., ZHANG, Y., WU, Q., CUI, X., LIN, Z., LIU, S. & CHEN, L. 2014. Clinical implications of high NQO1 expression in breast cancers. *J Exp Clin Cancer Res,* 33**,** 14.

YUAN, J. M., CASTELAO, J. E., GAGO-DOMINGUEZ, M., YU, M. C. & ROSS, R. K. 1998. Tobacco use in relation to renal cell carcinoma. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology,* 7**,** 429-33.

YUSENKO, M. V., KUIPER, R. P., BOETHE, T., LJUNGBERG, B., VAN KESSEL, A. G. & KOVACS, G. 2009. High-resolution DNA copy number and gene expression analyses distinguish chromophobe renal cell carcinomas and renal oncocytomas. *BMC Cancer,* 9**,** 152.

ZAGER, R. A., JOHNSON, A. C. & LUND, S. 2007. 'Endotoxin tolerance': TNF-alpha hyper-reactivity and tubular cytoresistance in a renal cholesterol loading state. *Kidney Int,* 71**,** 496-503.

ZAJAC-KAYE, M. 2001. Myc oncogene: a key component in cell cycle regulation and its implication for lung cancer. *Lung Cancer,* 34 Suppl 2**,** S43-6.

ZAMAN, U. K. S., KHALIL, M., RAHMAN, M. M., ARA, Z. G., AFRIN, S., SULTANA & Z, R. 2011. Histological Changes of Human Kidney with Age in Bangladeshi People. *Bangladesh Medical Journal,* 40**,** 13-17.

ZAPPITELLI, G. & 2009. *Acute Kidney Injury: General Aspects,* Berlin:, Pediatric Nephrology in the Icu. Berlin:Springer.

ZAPPITELLI, M., WASHBURN, K. K., ARIKAN, A. A., LOFTIS, L., MA, Q., DEVARAJAN, P., PARIKH, C. R. & GOLDSTEIN, S. L. 2007. Urine neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in critically ill children: a prospective cohort study. *Crit Care,* 11**,** R84.

ZBAR, B. 2000. Inherited epithelial tumors of the kidney: old and new diseases. *Semin Cancer Biol,* 10**,** 313-8.

ZBAR, B., KISHIDA, T., CHEN, F., SCHMIDT, L., MAHER, E. R., RICHARDS, F. M., CROSSEY, P. A., WEBSTER, A. R., AFFARA, N. A., FERGUSON-SMITH, M. A., BRAUCH, H., GLAVAC, D., NEUMANN, H. P., TISHERMAN, S., MULVIHILL, J. J., GROSS, D. J., SHUIN, T., WHALEY, J., SEIZINGER, B., KLEY, N., OLSCHWANG, S., BOISSON, C., RICHARD, S., LIPS, C. H., LERMAN, M. & ET AL. 1996. Germline mutations in the Von Hippel-Lindau disease (VHL) gene in families from North America, Europe, and Japan. *Hum Mutat,* 8**,** 348-57.

ZENG, F., SINGH, A. B. & HARRIS, R. C. 2009. The role of the EGF family of ligands and receptors in renal development, physiology and pathophysiology. *Exp Cell Res,* 315**,** 602-10.

ZGODA-POLS J. R., CHOWDHURY, S., WIRTH, M., MILBURN, M.V., ALEXANDER, D.C. & ALTON K. B. 2011. Metabolomics analysis reveals elevation of 3-indoxyl sulfate in plasma and brain during chemically-induced acute kidney injury in mice: investigation of nicotinic acid receptor agonists. Toxicol Appl Pharmacol, 255, 48–56.

ZHANG, X., LIU, S., GUO, C., ZONG, J. & SUN, M. Z. 2012. The association of annexin A2 and cancers. *Clin Transl Oncol,* 14**,** 634-40.

ZHAO, S. H., RECKNOR, J., LUNNEY, J. K., NETTLETON, D., KUHAR, D., ORLEY, S. & TUGGLE, C. K. 2005. Validation of a first-generation long-oligonucleotide microarray for transcriptional profiling in the pig. *Genomics,* 86**,** 618-25.

ZHONG, L. P., WEI, K. J., YANG, X., ZHANG, L., ZHOU, X. J., PAN, H. Y., LI, J., CHEN, W. T. & ZHANG, Z. Y. 2009. Increased expression of Annexin A2 in oral squamous cell carcinoma. *Arch Oral Biol,* 54**,** 17-25.

ZUK, A. & BONVENTRE, J. V. 2016. Acute Kidney Injury. *Annu Rev Med,* 67**,** 293-307.

ZUK, A., BONVENTRE, J. V., BROWN, D. & MATLIN, K. S. 1998. Polarity, integrin, and extracellular matrix dynamics in the postischemic rat kidney. *Am J Physiol,* 275**,** C711-31.

ANNEXURE

I. How to download data from SRA using command line

```
#!/bin/bash
nohup
wgetftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/000/SRR1144040/SRR1144040.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/001/SRR1144041/SRR1144041.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/002/SRR1144042/SRR1144042.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/003/SRR1144043/SRR1144043.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/004/SRR1144044/SRR1144044.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/005/SRR1144045/SRR1144045.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/006/SRR1144046/SRR1144046.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/007/SRR1144047/SRR1144047.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/008/SRR1144048/SRR1144048.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/009/SRR1144049/SRR1144049.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/000/SRR1144050/SRR1144050.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/001/SRR1144051/SRR1144051.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/002/SRR1144052/SRR1144052.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/003/SRR1144053/SRR1144053.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/004/SRR1144054/SRR1144054.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/005/SRR1144055/SRR1144055.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/006/SRR1144056/SRR1144056.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/007/SRR1144057/SRR1144057.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/008/SRR1144058/SRR1144058.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/009/SRR1144059/SRR1144059.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/000/SRR1144060/SRR1144060.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/001/SRR1144061/SRR1144061.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/002/SRR1144062/SRR1144062.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/003/SRR1144063/SRR1144063.fastq.gz;
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wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/008/SRR1144068/SRR1144068.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/009/SRR1144069/SRR1144069.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/000/SRR1144070/SRR1144070.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/001/SRR1144071/SRR1144071.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/002/SRR1144072/SRR1144072.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/003/SRR1144073/SRR1144073.fastq.gz;
wget ftp://ftp.sra.ebi.ac.uk/vol1/fastq/SRR114/004/SRR1144074/SRR1144074.fastq.gz;
```

II. Alignments by Tophat (using iGenomes annotation and sequence files) and DEG calculation by Cufflinks, Cuffdiff

tophat -p7 -o Ctrl43261 genome Ctrl4326_1.fastq.gz;
tophat -p7 -o Ctrl43262 genome Ctrl4326_2.fastq.gz;
tophat -p7 -o Ctrl43501 genome Ctrl4350_1.fastq.gz;
tophat -p7 -o Ctrl43502 genome Ctrl4350_2.fastq.gz;
tophat -p7 -o Ctrl43761 genome Ctrl4376_1.fastq.gz;
tophat -p7 -o Ctrl43762 genome Ctrl4376_2.fastq.gz;
tophat -p7 -o HIF1A4329_1 genome HIF1A4329_1.fastq.gz;
tophat -p7 -o HIF1A4329_2 genome HIF1A4329_2.fastq.gz;
tophat -p7 -o HIF1A4349_1 genome HIF1A4349_1.fastq.gz;
tophat -p7 -o HIF1A4349_2 genome HIF1A4349_2.fastq.gz;
tophat -p7 -o HIF1A4375_1 genome HIF1A4375_1.fastq.gz;
tophat -p7 -o HIF1A4375_2 genome HIF1A4375_2.fastq.gz;

cufflinks -p 7 -o newCtrl43261cuff -g genes.gtf Ctrl43261_accepted_hits.bam;
cufflinks -p 7 -o newCtrl43262cuff -g genes.gtf Ctrl43262_accepted_hits.bam;
cufflinks -p 7 -o newCtrl43501cuff -g genes.gtf Ctrl43501_accepted_hits.bam;
cufflinks -p 7 -o newCtrl43502cuff -g genes.gtf Ctrl43502_accepted_hits.bam;
cufflinks -p 7 -o newCtrl43761cuff -g genes.gtf Ctrl43761_accepted_hits.bam;
cufflinks -p 7 -o newCtrl43762cuff -g genes.gtf Ctrl43762_accepted_hits.bam;
cufflinks -p 7 -o newHIF1A4329_1cuff -g genes.gtf HIF1A43291_accepted_hits.bam;
cufflinks -p 7 -o newHIF1A4329_2cuff -g genes.gtf HIF1A43292_accepted_hits.bam;
cufflinks -p 7 -o newHIF1A4349_1cuff -g genes.gtf HIF1A43491_accepted_hits.bam;
cufflinks -p 7 -o newHIF1A4349_2cuff -g genes.gtf HIF1A43492_accepted_hits.bam;
cufflinks -p 7 -o newHIF1A4375_1cuff -g genes.gtf HIF1A43751_accepted_hits.bam;
cufflinks -p 7 -o newHIF1A4375_2cuff -g genes.gtf HIF1A43752_accepted_hits.bam;

cuffdiff -o trackcuffdiff -p 8 genes.gtf Ctrl43261_accepted_hits.bam,Ctrl43262_accepted_hits.bam,Ctrl43501_accepted_hits.bam,Ctrl43502_accepted_hits.bam,Ctrl43761_accepted_hits.bam,Ctrl43762_accepted_hits.bam HIF1A43291_accepted_hits.bam,HIF1A43292_accepted_hits.bam,HIF1A43491_accepted_hits.bam,HIF1A43492_accepted_hits.bam,HIF1A43751_accepted_hits.bam,HIF1A43752_accepted_hits.bam

III. Alignment and DEG calculation by RSEM

rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam HIF1A4329_1.fastq.gz mouse_ref_rsem/mouse_ref rsem/hif1a43291;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam HIF1A4329_2.fastq.gz mouse_ref_rsem/mouse_ref rsem/hif1a43291;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam HIF1A4349_1.fastq.gz mouse_ref_rsem/mouse_ref rsem/hif1a43491;

rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam HIF1A4349_2.fastq.gz mouse_ref_rsem/mouse_ref rsem/hif1a43492;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam HIF1A4375_1.fastq.gz mouse_ref_rsem/mouse_ref rsem/hif1a43751;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam HIF1A4375_2.fastq.gz mouse_ref_rsem/mouse_ref rsem/hif1a43752;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam Ctrl4326_1.fastq.gz mouse_ref_rsem/mouse_ref rsem/ctrl43261;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam Ctrl4326_2.fastq.gz mouse_ref_rsem/mouse_ref rsem/ctrl43262;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam Ctrl4376_1.fastq.gz mouse_ref_rsem/mouse_ref rsem/ctrl43761
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam Ctrl4376_2.fastq.gz mouse_ref_rsem/mouse_ref rsem/ctrl43762;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam Ctrl4350_1.fastq.gz mouse_ref_rsem/mouse_ref rsem/ctrl43751;
rsem-calculate-expression -p 6 --bowtie2 --estimate-rspd --append-names --output-genome-bam Ctrl4350_2.fastq.gz mouse_ref_rsem/mouse_ref rsem/ctrl43752;

IV. MapSplice

python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/HIF1A4375_1.fastq -o hif1a43751mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/HIF1A4375_2.fastq -o hif1a43752mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/HIF1A4329_1.fastq -o hif1a43291mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/HIF1A4329_2.fastq -o hif1a43292mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/HIF1A4349_1.fastq -o hif1a43491mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/HIF1A4349_2.fastq -o hif1a43492mapsplice;

python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/Ctrl4376_1.fastq -o ctrl43761mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/Ctrl4376_2.fastq -o ctrl43762mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/Ctrl4350_1.fastq -o ctrl43501mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/Ctrl4350_2.fastq -o ctrl43502mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/Ctrl4326_1.fastq -o ctrl43261mapsplice;
python /home/svznpm/MapSplice-v2.1.8/mapsplice.py -c /home/svznpm/MapSplice-v2.1.8/chromFa -x /home/svznpm/MapSplice-v2.1.8/bowtie_index/built_bowtie_index -1 /media/svznpm/Data2/ngs/Mus_musculus_Ensembl_GRCm38/Ctrl4326_2.fastq -o ctrl43262mapsplice;

v. Alignment with BWA

bwa mem -t 7 musbwaidx Ctrl4326_1.fastq.gz > Ctrl4326_1.sam;
bwa mem -t 7 musbwaidx Ctrl4326_2.fastq.gz > Ctrl4326_2.sam;
bwa mem -t 7 musbwaidx Ctrl4350_1.fastq.gz > Ctrl4350_1.sam;
bwa mem -t 7 musbwaidx Ctrl4350_2.fastq.gz > Ctrl4350_2.sam;
bwa mem -t 7 musbwaidx Ctrl4376_1.fastq.gz > Ctrl4376_1.sam;
bwa mem -t 7 musbwaidx Ctrl4376_2.fastq.gz > Ctrl4376_2.sam;
bwa mem -t 7 musbwaidx HIF1A4329_1.fastq.gz > HIF1A4329_1.sam;
bwa mem -t 7 musbwaidx HIF1A4329_2.fastq.gz > HIF1A4329_2.sam;
bwa mem -t 7 musbwaidx HIF1A4375_1.fastq.gz > HIF1A4375_1.sam;
bwa mem -t 7 musbwaidx HIF1A4375_2.fastq.gz > HIF1A4375_2.sam;
bwa mem -t 7 musbwaidx HIF1A4349_1.fastq.gz > HIF1A4349_1.sam;
bwa mem -t 7 musbwaidx HIF1A4349_2.fastq.gz > HIF1A4349_2.sam;

6. SNV identification for Tophat2 and BWA alignment files

a. SAM files generated by BWA alignments are convered to SAM format using Samtool view

samtools view -bS input.sam > input.bam;

b. BAM files are sorted using Samtools sort

samtools sort input.bam input.sort.bam

c. Samtools rmdup used to remove duplucats

samtools rmdup -s input.sort.bam input.sort.rmdup.bam

d. Samtools mpileup used to identify variants relative to reference genome

samtools mpileup -g -f genome.fa input.sort.rmdup.bam > input.sort.rmdup.raw.bcf

e. Bcftools used to filter

bcftools view -bvcg input.sort.rmdup.raw.bcf > input.sort.rmdup.var.bcf

f. Bcftools used to convert bcf to vcf format

bcftools view HIF1A4329_1.sort.rmdup.var.bcf vcfutils.pl varFilter - > HIF1A4329_1.sort.rmdup.varbcf.vcf

g. vcfintersect (from VCFlib) used to intersect vcf files from Control and TRACK respectively

vcfintersect -i ctrl4326.sort.rmdup.varbcf.vcf ctrl4376.sort.rmdup.varbcf.vcf -r genome.fa > ctrl4326.vcf

http://eprints.nottingham.ac.uk/id/eprint/40640